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VOLUME 3 NOVEMBER, 1930 NUMBER 5

PHYSICO-CHEMICAL STUDIES ON THE NATURE OF DROUGHT RESISTANCE IN CROP PLANTS[1]

BY R. NEWTON AND W. M. MARTIN

VI. Properties of Plant-tissue Fluids in Relation to Drought Resistance

The osmotic pressure and bound water of the tissue fluids of various crop plants and some others of interest were determined at intervals during the three seasons of 1924-25-26. In the last season only, the electrical conductivity, hydrogen ion concentration, colloidal content and gold number were added to the list of properties investigated. The results for each season will be presented separately and briefly discussed, followed at the end of the section by a general discussion of the whole.

EXPERIMENTS OF 1924

The concentration, osmotic pressure and bound water of all juice samples subjected to routine examination during the season of 1924 are assembled in Table XVII. It will be remembered that the concentration as read on the sugar scale of the refractometer was during this season reduced by 15.3%, a correction which towards the end of the season was found too small (Table V). The negative values for bound water which resulted in a few cases indicate that the water content of the juice was underestimated. Probably most of the bound-water readings are slightly low for the same reason.

The bound water in the juices examined up to June 28, marked in the table with an asterisk, were determined with dextrose instead of sucrose. The inferiority of the former sugar for this purpose had by that time been demonstrated by a special experiment (Table I) and sucrose was used in all subsequent work. This change of method unfortunately breaks the continuity of the series of results obtained during that season.

Another factor which militates against the significance of the results is the late date of the last collections. It was found that the various stages of maturity exhibited by the different species tended to invalidate the comparisons. With the approach of maturity the moisture content of the tissues decreases rapidly, causing corresponding increases in concentration and bound water of the juice, which are not necessarily related to drought resistance.

[1] *Concluded from October issue.*

TABLE XVII

CONCENTRATION, OSMOTIC PRESSURE AND BOUND WATER OF PRESS JUICE OF VARIOUS CROP PLANTS DURING THE SEASON OF 1924

Species	Date collected 1924	Conc'n by refract. %	Osmotic pressure atm.	Bound water %
Triticum compactum var. Hybrid 143	June 10	10.9	11.9	4.1*
	June 26	9.3	11.3	−0.4*
	July 21	16.7	16.2	4.7
	Aug. 20	21.7	16.4	10.9
T. vulgare var. Marquis	June 10	10.5	12.2	2.3*
	June 27	8.0	11.7	8.9*
	July 22	15.4	13.4	4.8
	Aug. 20	19.7	15.5	11.0
T. spelta var. White spelt	June 11	11.2	12.2	4.1*
	June 27	11.0	13.6	1.6*
	July 23	18.3	16.5	6.5
	July 24	18.6	17.0	7.4
	Aug. 21	20.4	17.1	12.4
T. turgidum var. Alaska	June 11	10.7	12.3	1.5*
	June 27	9.4	13.1	1.8*
	July 23	17.3	15.8	11.0
	Aug. 21	18.5	14.2	11.6
T. durum var. Kubanka	June 10	10.7	13.0	1.7*
	June 27	8.0	10.7	1.8*
	July 22	18.1	16.8	8.7
	Aug. 21	19.5	15.3	10.9
T. polonicum var. White Polish	June 10	11.6	12.6	2.2*
	June 27	9.6	11.7	1.9*
	July 22	16.3	14.8	7.6
	Aug. 21	16.2	13.0	9.1
T. dicoccum var. Common emmer	June 12	10.0	11.7	1.7*
	June 28	10.5	13.8	0.0*
	July 24	17.3	14.8	8.1
	Aug. 21	17.4	14.6	10.2
T. monococcum Einkorn	June 12	11.6	11.0	1.6*
	June 28	11.1	12.3	10.2*
	July 24	16.6	13.9	5.5
	Aug. 21	18.1	14.7	10.2
Avena sativa var. Banner	June 18	6.4	9.1	1.0*
	July 31	14.7	12.2	5.2
	Aug. 14	11.6	10.0	3.9
A. sativa var. Leader	June 18	6.7	9.1	1.9*
	July 31	15.2	12.7	5.5
	Aug. 14	12.6	10.8	4.4
A. nuda var. Chinese hulless	June 18	7.7	10.2	2.3*
	Aug. 1	10.7	9.6	2.3
	Aug. 15	11.9	9.9	5.0
A. fatua Wild oats	June 18	9.2	11.3	2.1*
	Aug. 1	10.3	9.8	3.2
	Aug. 12	11.9	9.1	3.8

TABLE XVII—*Continued*

CONCENTRATION, OSMOTIC PRESSURE AND BOUND WATER OF PRESS JUICE OF VARIOUS CROP PLANTS DURING THE SEASON OF 1924

Species	Date collected 1924	Conc'n by refract. %	Osmotic pressure atm.	Bound water %
A. sterilis Red oats	June 19	9.4	11.0	3.3*
	Aug. 1	14.5	12.0	4.5
	Aug. 15	17.0	13.5	7.1
A. sterilis var. *Ludoviciana*	June 19	8.5	10.9	2.9*
	Aug. 2	11.0	9.8	−0.1
	Aug. 16	12.0	11.0	4.8
A. strigosa Sand oats	June 19	9.0	8.9	2.6*
	Aug. 1	12.2	10.8	3.6
	Aug. 16	14.9	12.3	4.4
A. brevis Short oats	June 19	9.2	10.9	2.6*
	Aug. 2	11.1	9.1	−1.3
	Aug. 16	14.0	10.8	5.1
Hordeum vulgare var. O.A.C. No. 21	June 17	6.6	9.4	0.7*
	July 25	15.7	16.7	5.6
	Aug. 12	15.4	12.9	6.1
H. vulgare var. Barks	June 17	7.3	10.2	1.9*
	July 25	19.3	18.1	7.2
	Aug. 13	15.8	15.4	7.0
H. distichon var. Canadian Thorpe	June 17	6.4	9.9	2.2*
	July 25	14.1	15.4	3.2
	Aug. 13	14.1	13.4	8.7
H. distichon var. Hannchen	June 17	6.7	10.2	2.0*
	July 26	13.2	14.8	5.6
	Aug. 13	14.1	13.0	6.7
Secale cereale var. O.A.C. No. 62 (spring rye)	June 20	8.8	11.8	2.5*
	July 30	15.4	13.1	6.2
	Aug. 19	18.0	14.4	8.3
S. cereale var. Alberta winter	June 20	11.4	11.8	3.6*
	July 30	11.2	11.0	2.6
	Aug. 19	17.7	13.7	8.4
S. cereale var. Rosen (winter)	June 20	10.3	12.3	3.3*
	July 31	10.8	11.4	1.6
	Aug. 19	16.0	13.7	7.2
Agropyron tenerum Western rye grass	June 20	17.9	17.1	4.3*
	July 29	17.3	12.5	6.3
	Aug. 22	19.1	15.9	10.1
Phleum pratense Timothy	June 20	7.8	12.2	2.5*
	July 16	7.3	11.6	1.4
	July 29	5.7	8.9	0.5
	Aug. 22	5.3	8.8	0.5

TABLE XVII—*Continued*

CONCENTRATION, OSMOTIC PRESSURE AND BOUND WATER OF PRESS JUICE OF VARIOUS CROP PLANTS DURING THE SEASON OF 1924

Species	Date collected 1924	Conc'n by refract. %	Osmotic pressure atm.	Bound water %
Zea mays var. Northwestern dent	Sept. 2	7.8	7.5	4.6
Helianthus annuus var. Russian giant	Sept. 2	8.0	8.3	3.1
AVERAGES, in order of magnitude†				
Wheat		18.0	15.3	8.9
Spring rye		16.7	13.7	7.2
Barley		15.2	15.0	6.3
Oats		12.8	10.8	3.8
Western rye grass		18.2	14.2	8.2
Timothy		6.1	9.8	0.8

Coefficients of correlation for wheat species

	n	*r*
Concentration and osmotic pressure	33	0.88 ± 0.03
Concentration and bound water†	17	0.65 ± 0.09
Osmotic pressure and bound water†	17	0.17 ± 0.16

**Dextrose used in determination.*
†*Including only collections in which bound water determined with sucrose.*

With the spring cereals, seeded at the usual time under Edmonton conditions, comparable results can be secured only up to about July 20. The experimental season was lengthened the next two years by making second seedings at later dates.

Still another handicap to the interpretation of the results is the variability of properties in different collections. In one variety, white spelt, collections were made on two succeeding days, July 23 and 24, and it will be seen that in the interval an appreciable increase had taken place in the value of all three properties measured, most pronounced in the bound-water content. The solution of this difficulty in subsequent seasons was sought in a reduction of the number of crops studied, in order to permit more frequent collections and secure a more reliable average.

Defects of the foregoing character are perhaps not surprising in the results of the first season's determinations, especially as the experiments described in Sections III and IV, relating to the theory and technique of the methods employed, were done not in advance but concurrently. These defects, however, while they interfere with the interpretation of the bound-water values in

relation to drought resistance, do not prevent an interesting comparison of the relationship between concentration of juice, osmotic pressure and bound water.

Osmotic pressure tends in general to increase with the progress of maturity, but not proportionally as much as the concentration of the juice. This suggests that cell colloids may be elaborated more abundantly in the latter part of the season. While it is more difficult to discern the trend of the bound-water values, because of the use of two methods in their determination, if we confine comparisons to those values found with sucrose we see that they increased rapidly towards the end of the season, in this case proportionally more than the concentration of the juice. This would be expected from a consideration of the fact that as the ratio of water to colloid diminishes, the percentage of the water bound increases. This is true notwithstanding the fact that the water bound per gram of colloid normally decreases with concentration.

The coefficient of correlation r between the properties measured, and the number of cases n on which they are based, are given for the wheat species at the end of the table. They were limited to the wheat species to make the figures comparable with those obtained in succeeding years, when the other cereals were not studied. There may also be a question as to how far it is legitimate to include different genera in the same correlation table. An inspection of the figures for the oat species indicates a lower average hydration of the dry substance than was found in the wheats. This may be the result of a characteristically lower ratio of colloids to crystalloids in the tissue fluids of oats, which would obviously disturb the correlation of, for example, concentration and osmotic pressure, in any table including both wheat and oats. To avoid another disturbing factor, where bound water is concerned, only the determinations made with sucrose were included in the calculations.

It must be remembered that the Pearson coefficient r indicates the degree of linear relationship. A relation involving the hydration of colloids is likely to be curved (Fig. 3). On the other hand, the relation between concentration and osmotic pressure is likely to be linear (Fig. 4). Furthermore, bound water is affected by hydrogen ion concentration and by the state of dispersion of the colloids, factors which have little influence on osmotic pressure. These differences probably account largely for the greater magnitude of the correlation between concentration of juice and osmotic pressure. The fact that it is not perfect indicates variation in average particle size, no doubt due in part to variation in colloidal content. The absence of apparent correlation between osmotic pressure and bound water in the 1924 results makes it necessary to conclude that, while both of these quantities may be related to drought resistance, they cannot both be a direct index of this property.

While the results of the 1924 work do not enable us in most cases to classify individual species or varieties according to drought resistance, the averages for the different classes of cereals are not without significance in this regard. These are given towards the end of Table XVII, the figures being based on the collections in which bound water was determined with sucrose. Wheat,

spring rye, barley and oats fall in this order in magnitude of juice concentration, osmotic pressure (with one exception) and bound water. This conforms closely to the known adaptations of these plants. Possibly wheat and rye should be reversed in position; the present classification of spring rye rests upon the evidence of one variety, the winter varieties being excluded because they were planted in the spring and did not advance towards maturity like all the other cereals included in the tests. The percentage of spread between the cereals is greatest in bound water, suggesting this as the most sensitive indicator of differences in drought adaptation.

In at least one case there is a clear-cut relationship between bound water and known drought resistance. This is seen in a comparison of the figures for western rye grass and timothy, included in the averages and given in detail just above. In this case the latest collection of leaves was made from second-growth plants, and the values are therefore not affected by the onset of maturity. Western rye grass is drought-hardy and well adapted to the dry areas of the prairie provinces. Timothy is a widely-grown hay plant in other parts of America, but is distinctly not drought-hardy. The juice of the western rye leaves was more concentrated than that of the timothy leaves at all dates of collection, and higher in both osmotic pressure and bound water. The especially striking differences in their bound-water contents point again to the utility of this property in distinguishing drought-resistant plants.

Experiments of 1925

In 1925 the investigations of tissue fluids were restricted to wheats and grasses. The same data were obtained as in 1924, but in more complete and satisfactory series. The concentration as read on the sugar scale of the refractometer was reduced by a correction of 17.0%; all bound-water determinations were carried out with sucrose; all tissue collected was in a satisfactory condition of immaturity and succulence; and enough collections were made of important cultivated species to get dependable averages.

Two seedings were made of the wheats, to lengthen the period over which suitable leaf material could be collected. Timothy, western rye grass, and western wheat grass were collected from plots seeded May 13 of the same season (except the first collection of timothy and western rye, which was from plots seeded the previous year). The other cultivated grasses were collected from carefully selected typical habitats which will be described below. More collections were made of timothy and western rye than of the other grasses, because of their special importance and of the striking nature of the results they had given the previous season.

The 1925 data for the wheats and grasses respectively are presented in Tables XVIII and XIX. The seasonal trends in the values for concentration, osmotic pressure and bound water are not so marked as in 1924, because of the avoidance of maturing tissues in 1925, but may still be discerned in some cases. As before, fluctuations occur owing to weather conditions. The average bound-water values, however, enable us to classify the species and varieties very satisfactorily in regard to drought resistance. This point will

TABLE XVIII

CONCENTRATION, OSMOTIC PRESSURE AND BOUND WATER OF PRESS JUICE OF WHEAT LEAVES DURING SEASON OF 1925

Species	Date seeded 1925	Date collected 1925	Conc'n by refract. %	Osmotic pressure atm.	Bound water %
Triticum compactum var. Hybrid 143	May 13	June 26	12.9	11.8	6.5
	May 13	July 2	14.5	13.3	5.0
	June 22	July 20	12.3	13.6	6.2
	June 22	July 28	12.9	12.7	4.6
	June 22	Aug. 3	11.4	12.4	5.3
Average			12.8	12.8	5.5
T. vulgare var. Marquis	May 13	June 24	13.8	12.2	2.0
	May 13	June 30	17.8	17.7	5.3
	June 22	July 20	14.5	13.9	5.2
	June 22	July 28	16.6	13.6	4.1
	June 22	Aug. 3	17.1	15.9	2.7
Average			15.9	14.6	3.9
T. vulgare var. Caesium	May 13	June 26	15.3	13.9	7.0
	May 13	June 30	18.2	17.0	8.3
	June 22	July 28	12.9	12.9	6.1
	June 22	Aug. 5	10.7	11.7	5.2
Average			14.3	13.9	6.7
T. vulgare var. Crimka	May 13	June 27	11.5	13.3	3.3
	May 13	July 3	12.6	13.6	6.1
Average			12.0	13.5	4.7
T. spelta var. White spelt	May 13	June 26	15.3	14.3	4.9
	May 13	July 2	18.7	16.2	5.7
	June 22	July 28	14.1	12.8	6.8
	June 22	Aug. 5	15.3	13.6	5.5
Average			15.8	14.2	5.7
T. turgidum var. Alaska	May 13	June 26	12.8	12.4	5.4
	May 13	July 2	15.8	14.4	6.3
	June 22	July 20	13.2	13.0	6.9
	June 22	July 29	13.1	12.6	6.1
	June 22	Aug. 3	14.2	14.9	7.2
Average			13.8	13.3	6.4
T. durum var. Kubanka	May 13	June 24	12.2	11.2	4.9
	May 13	June 30	17.0	15.8	8.2
	June 22	July 20	13.2	13.8	6.1
	June 22	July 29	14.9	12.4	5.5
	June 22	Aug. 3	16.6	16.1	9.0
Average			14.8	13.9	6.7
T. polonicum var. White Polish	May 13	June 26	12.1	11.9	4.9
	May 13	July 2	15.9	14.8	7.1
	June 22	July 22	12.4	12.7	1.4
	June 22	July 29	12.4	12.3	3.4
	June 22	Aug. 4	11.8	13.3	4.2
Average			12.9	13.0	4.2

TABLE XVIII—*Continued*

CONCENTRATION, OSMOTIC PRESSURE AND BOUND WATER OF PRESS JUICE OF WHEAT LEAVES DURING THE SEASON OF 1925

Species	Date seeded 1925	Date collected 1925	Conc'n by refract. %	Osmotic pressure atm.	Bound water %
T. dicoccum var. Common emmer	May 13	June 27	15.6	13.6	6.1
	May 13	June 30	17.8	16.1	7.9
	June 22	July 22	13.8	12.5	3.9
	June 22	July 29	17.3	14.4	7.7
	June 22	Aug. 4	17.8	15.0	9.8
Average			16.4	14.3	7.1
T. monococcum Einkorn	May 13	June 27	14.4	12.9	4.1
	May 13	July 3	15.3	13.5	7.2
	June 22	July 28	14.2	11.5	5.0
	June 22	Aug. 5	15.6	13.2	5.4
Average			14.9	12.7	5.4

Coefficients of correlation

	n	*r*
Concentration and osmotic pressure	44	0.81 ± 0.03
Concentration and bound water	44	0.44 ± 0.08
Osmotic pressure and bound water	44	0.43 ± 0.08

TABLE XIX

CONCENTRATION, OSMOTIC PRESSURE AND BOUND WATER OF PRESS JUICE OF GRASSES DURING SEASON OF 1925

Species	Date collected	Conc' by refract. %	Osmotic pressure atm.	Bound water %
CULTIVATED GRASSES				
Phleum pratense Timothy	June 17	9.3	12.9	2.0
	June 29	8.4	13.7	3.5
	July 3	9.3	14.2	3.0
	July 7	9.5	18.8	9.2
	July 17	10.0	15.1	2.3
	July 30	12.0	17.9	−0.8
	Aug. 5	11.7	17.5	4.7
Average		10.0	15.7	3.4
Agropyron tenerum Western rye grass	June 17	16.8	15.9	8.0
	June 29	16.8	17.1	6.5
	July 3	16.7	15.8	6.5
	July 7	21.2	21.6	13.6
	July 17	14.2	17.8	12.2
	July 30	17.3	20.1	11.9
	Aug. 4	18.2	22.5	14.8
Average		17.3	18.7	10.5

TABLE XIX—*Continued*

CONCENTRATION, OSMOTIC PRESSURE AND BOUND WATER OF PRESS JUICE OF GRASSES DURING SEASON OF 1925

Species	Date collected 1925	Conc' by refract. %	Osmotic pressure atm.	Bound water %
Agropyron cristatum Crested wheat grass	June 29	18.4	20.8	11.7
	July 3	18.3	18.8	10.8
	July 7	20.5	21.1	12.6
Average		19.1	20.3	11.7
Agropyron smithii Western wheat grass	July 17	14.9	15.4	7.4
	July 29	17.4	17.4	5.8
	Aug. 5	17.5	19.3	8.1
Average		16.6	17.4	7.1
Poa pratensis Kentucky blue grass	July 9	9.5	13.4	4.7
Bromus inermis Awnless brome grass	July 9	14.3	19.7	10.6
WILD GRASSES				
Bouteloua gracilis Blue grama grass	July 8	22.6	34.9	16.7
Stipa comata Western spear grass	July 10	17.2	23.2	15.1
Calamovilfa longifolia Sand grass	July 13	9.0	13.2	6.9
Beckmannia erucaeformis Slough grass	July 14	10.4	11.3	3.1
Panicularia grandis Tall manna grass	July 14	9.5	9.7	4.4
Fluminea festucacea Prickle fescue grass	July 14	12.8	13.4	4.3
Calamagrostis canadensis Bluejoint grass	July 16	8.5	11.9	3.7

Coefficients of correlation

	n	*r*
Concentration and osmotic pressure	29	0.78±0.05
Concentration and bound water	29	0.78±0.05
Osmotic pressure and bound water	29	0.79±0.05

be deferred for consideration together with the 1926 results. The discussion of the coefficients of correlation between concentration, osmotic pressure and bound water, added at the ends of the tables, will also be deferred.

In collecting the wild grasses, as intimated above, ecological relationships were taken into account. They were taken from habitats in which their relative abundance showed them to be well adapted. *Bouteloua gracilis*, *Stipa*

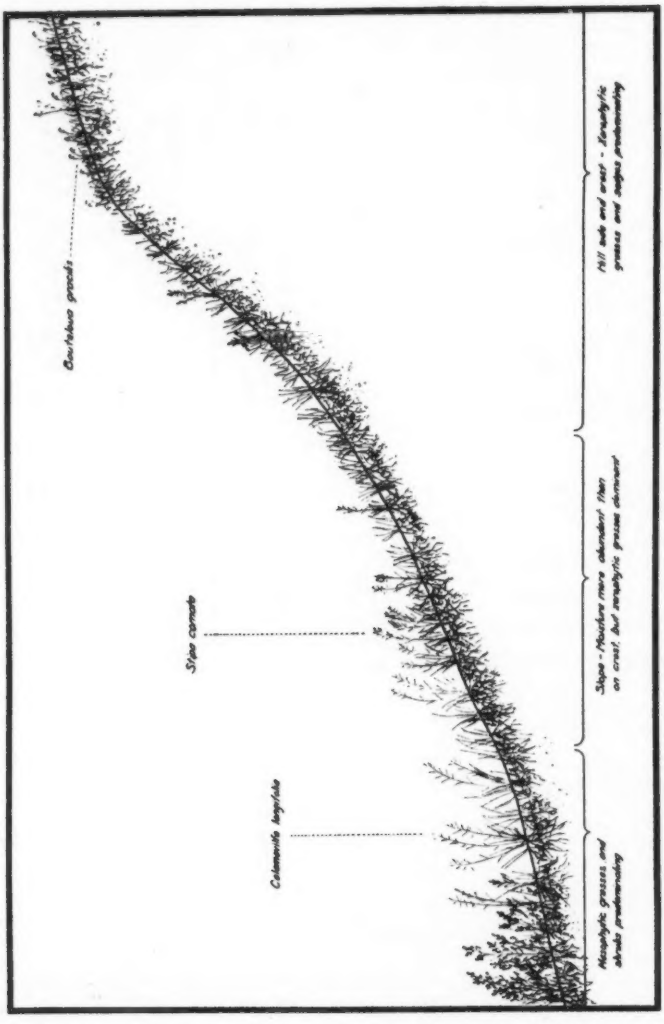


FIG. 14. *Line transect of hillside on north bank of Saskatchewan river at Edmonton, showing plants collected for laboratory study. Linear scale, 1:600.*

comata, and *Calamovilfa longifolia* were obtained from a dry hillside on the north bank of the Saskatchewan river, at Edmonton; while *Beckmannia erucaeformis*, *Panicularia grandis*, and *Fluminea festucacea* were collected in or around a slough about six miles east of Edmonton. Line transects of these two habitats were run, and the vegetation carefully charted. The transects shown in

TABLE XX

LINE TRANSECT OF VEGETATION ON HILLSIDE (FIG. 14)

Distance along line from crest in ft.	Species
40	*Bouteloua gracilis* (H.B.K.) Lag.
	Stipa viridula Trin.
	Symphoricarpos racemosus Michx.
50	*Carex filifolia* Nutt.
	Bouteloua gracilis (H.B.K.) Lag.
	Astragalus pectinatus Dougl.
60	*Carex filifolia* Nutt.
	Stipa viridula Trin.
	Artemisia gnaphalodes Nutt.
	Bouteloua gracilis (H.B.K.) Lag. (dominant)*
70	*Carex filifolia* Nutt.
	Stipa viridula Trin.
	Astragalus pectinatus Dougl.
	Stipa comata Trin. & Rup.
	Stipa viridula Trin.
80	*Rosa* sp.
	Carex filifolia Nutt.
90	*Bouteloua gracilis* (H.B.K.) Lag.
	Stipa viridula Trin.
	Astragalus pectinatus Dougl.
	Stipa comata Trin. & Rup.
	Carex sp.
120	*Bouteloua gracilis* (H.B.K.) Lag.
	Stipa viridula Trin.
	Artemisia gnaphalodes Nutt.
	Symphoricarpos racemosus Michx.
130	*Carex filifolia* Nutt.
	Bouteloua gracilis (H.B.K.) Lag.
	Stipa comata Trin. & Rup.*
140	*Carex* sp.
	Bouteloua gracilis (H.B.K.) Lag.
	Artemisia gnaphalodes Nutt.
	Rosa sp.
150	*Stipa comata* Trin. & Rup.
	Calamovilfa longifolia (Hook.) Hack.
	Koeleria gracilis Pers.
	Artemisia gnaphalodes Nutt.
160	*Calamovilfa longifolia* (Hook.) Hack.*
	Rosa sp.
	Stipa viridula Trin.
	Symphoricarpos racemosus Michx.
170	*Poa pratensis* L.
	Koeleria gracilis Pers.
	Populus candicans Michx.
	Poa pratensis L.
180	*Symphoricarpos racemosus* Michx.
	Populus candicans Michx.
	Poa pratensis L. (dominant)
	Artemisia gnaphalodes Nutt.

*Leaves collected for laboratory study.

Fig. 14 and 15 are accompanied by ordered lists, in Tables XX and XXI, of the vegetation composing the two communities. If the two lists are taken in sequence, they run the gamut from xerophytism at the crest of the hill in the first transect to hydrophytism in the submerged portion of the slough in the

second. Between the two, but closer to the slough in moisture conditions, lies a third habitat, the woodland shown in Plate I-A, from which was collected

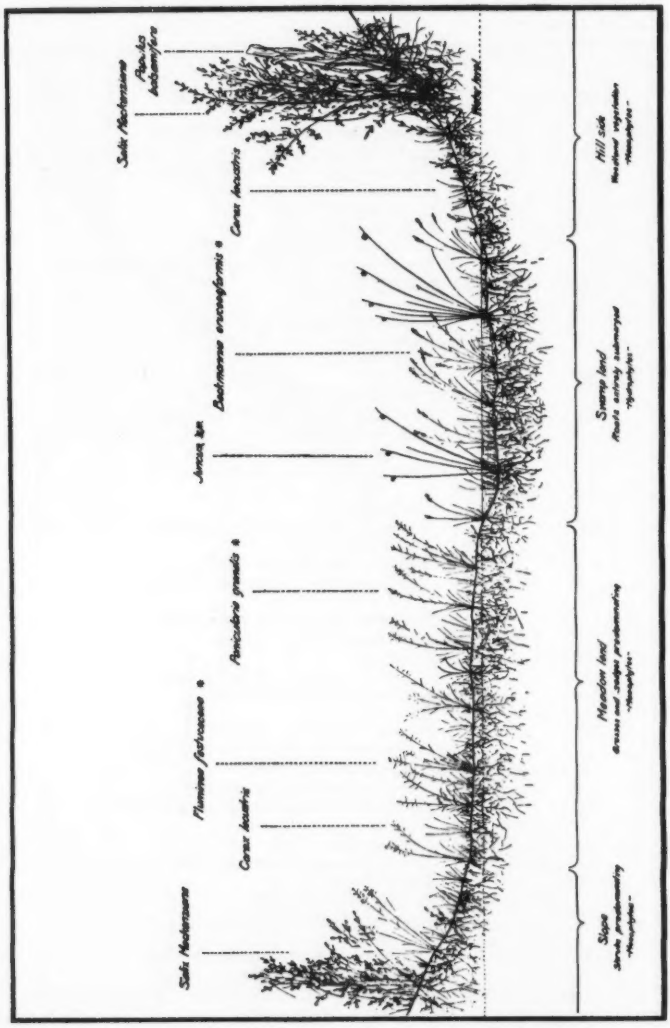


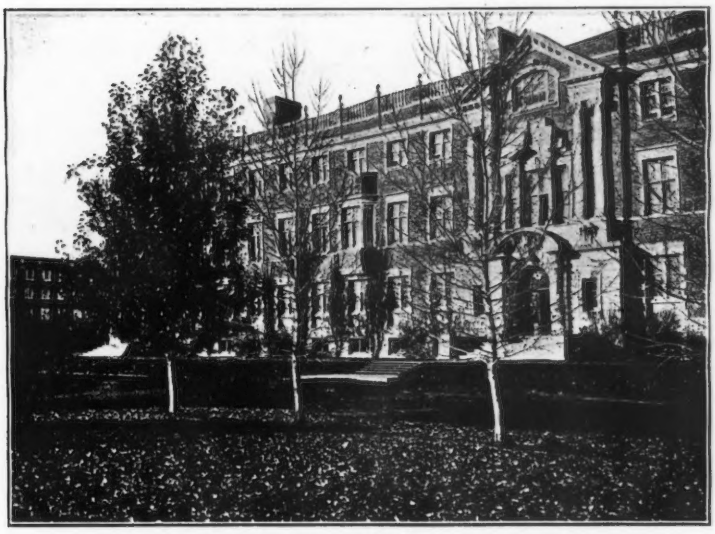
FIG. 15. *Line transect of slough in vicinity of Edmonton, showing plants collected for laboratory study. Linear scale, 1:300.*

Calamagrostis canadensis. It will be seen later that a knowledge of the habitat is of great assistance in interpreting the properties of the tissue fluids.

EXPERIMENTS OF 1926

In 1926, as already stated, the work was extended to include a number of additional properties, and these were determined on both fresh and dialysed juice. In Tables XXII and XXIII, which contain the results with wheats and

A. *Woodland habitat showing Calamagrostis canadensis (Michx.) Beauv.*



B. *Poplar trees on campus of University of Alberta. Leaves retained on Populus certinensis; leaves fallen from P. petrovski. (Photo Oct. 8, 1926.)*

TABLE XXI

LINE TRANSECT OF VEGETATION IN AND AROUND SLOUGH (FIG. 15)

Elevation from starting point in ft.	Distance along line from left in ft.	Species
−0.80	90	*Carex lacustris* Willd. (dominant) *Mentha spicata* L. *Calamagrostis rubescens* Buckley
−1.01	100	*Carex lacustris* Willd. (dominant) *Fluminea festucacea* (Willd.) Hitchc. *Carex lacustris* Willd.
−1.18	110	*Fluminea festucacea* (Willd.) Hitchc. *Carex lacustris* Willd. *Galium trifidum* L. *Mentha spicata* L.
−1.28	120	*Fluminea festucacea* (Willd.) Hitchc.* *Juncus* sp. *Cicuta maculata* L. *Fluminea festucacea* (Willd.) Hitchc. *Galium trifidum* L.
−1.49	130	*Fluminea festucacea* (Willd.) Hitchc. *Calamagrostis rubescens* Buckley *Carex lacustris* Willd. (dominant) *Fluminea festucacea* (Willd.) Hitchc. *Juncus* sp. *Cicuta maculata* L.
−1.71	140	*Carex lacustris* Willd. (dominant) *Panicularia grandis* (S.Wats.) Nash (dominant)* *Juncus* sp. *Beckmannia erucaeformis* (L.) Host
−1.60	150	*Panicularia grandis* (S.Wats.) Nash (dominant) *Juncus* sp. *Beckmannia erucaeformis* (L.) Host
−1.77	160	*Juncus* sp. (dominant) *Beckmannia erucaeformis* (L.) Host (dominant)* *Panicularia grandis* (S.Wats.) Nash (sparse)
−1.71	170	*Spirodela polyrhiza* (L.) Schleid. *Beckmannia erucaeformis* (L.) Host *Juncus* sp. (dominant)
−1.72	190	*Spirodela polyrhiza* (L.) Schleid. *Juncus* sp. (dominant)
−1.68	200	*Spirodela polyrhiza* (L.) Schleid. (dominant) *Juncus* sp. *Spirodela polyrhiza* (L.) Schleid.

**Leaves collected for laboratory study.*

grasses respectively, only the properties of the fresh juice are shown for a few of the early collections. These are the cases in which dialyser sacs prepared with three coats of collodion were used, which sacs proved too impermeable for efficient dialysis (as noted in Section V), and the results were discarded as unrepresentative.

Approximately the same group of species was investigated in 1926 as in 1925 in order to make comparison possible. The wheats and cultivated grasses were collected from field plots as usual, the latter all from stands more than a year old, and the wild grasses from the same habitats as in 1925, described above. The labor of carrying out the dialyses and additional determinations,

TABLE XXII

PROPERTIES OF FRESH AND DIALYSED PRESS JUICE OF WHEAT LEAVES DURING SEASON OF 1926

Species	Date collected 1926	Conc'n by refract. %	Conc'n by drying %	Coagulation by dialysis %	Crystalloidal content %	Osmotic pressure atm.	Conductivity at 25° C. K×10⁶	Hydrogen ion conc'n pH	Gold number	Bound water %	Ratio colloids: crystalloids	Bound water per gm. colloid in gm	Per cent colloid / gold number
Triticum compactum var. Hybrid 143	June 15*												
Fresh juice		9.96	—	—	—	9.5	14.2	5.78	—	4.6	—	—	—
	June 29*												
Fresh juice		14.28	14.11	—	6.9	13.1	15.5	5.90	0.06	4.2	1.03	0.51	119.5
Dialysed 40 hr.		5.15	7.17	29.1	—	1.5	1.3	6.08	0.13	2.7	—	0.35	55.1
	July 20†												
Fresh juice		18.76	18.30	—	10.2	17.1	14.8	5.98	0.15	9.1	0.80	0.91	54.3
Dialysed 48 hr.		6.97	8.14	16.5	—	—	—	—	0.62	—	—	—	13.1
(diluted)		4.15	4.98	18.7	—	1.3	0.8	6.26	—	3.4	—	0.65	—
T. vulgare var. Marquis	June 14*												
Fresh juice		12.20	—	—	—	11.0	13.6	5.98	—	3.5	—	—	—
	June 22*												
Fresh juice		12.20	11.66	—	5.1	10.0	12.9	6.30	0.11	4.1	1.30	0.55	60.0
Dialysed 40 hr.		5.98	6.60	13.4	—	0.6	0.8	6.32	0.12	4.2	—	0.60	55.0
	July 6†												
Fresh juice		13.03	12.86	—	6.5	12.7	17.7	5.88	0.17	2.2	1.97	0.30	37.3
Dialysed 42 hr.		5.15	6.34	19.8	—	1.3	1.3	5.98	0.26	2.4	—	0.36	24.4
	July 21†												
Fresh juice		19.42	18.88	—	9.7	16.4	12.8	6.16	0.13	7.4	0.95	0.65	70.9
Dialysed 48 hrs.		8.22	9.22	13.3	—	—	—	—	0.41	—	—	—	22.5
(diluted)		4.15	4.70	14.2	—	0.5	0.6	6.29	—	3.4	—	0.68	—
T. vulgare var. Caesium	July 6†												
Fresh juice		12.03	12.29	—	5.5	12.4	18.4	6.10	0.12	3.1	1.25	0.40	56.8
Dialysed 42 hr.		5.89	6.82	11.7	—	1.0	0.9	6.15	0.31	3.2	—	0.44	22.0
	July 21†												
Fresh juice		19.34	18.68	—	11.1	16.6	15.2	6.22	0.05	9.8	0.68	1.05	151.2
Dialysed 48 hr.		6.39	7.56	18.4	—	—	—	—	0.27	—	—	—	28.0
(diluted)		4.15	4.85	17.3	—	0.6	0.6	6.30	—	4.1	—	0.80	—
T. spelta var. White spelt	June 26*												
Fresh juice		13.28	12.71	—	5.6	13.3	14.3	5.98	0.10	5.2	1.26	0.64	70.9
Dialysed 40 hr.		4.81	7.09	35.1	—	1.3	1.4	5.91	0.13	4.1	—	0.54	54.5
	July 20†												
Fresh juice		19.59	18.94	—	10.0	17.2	15.0	6.26	0.12	5.9	0.88	0.54	74.1
Dialysed 48 hr.		7.39	8.89	19.6	—	—	—	—	0.40	—	—	—	22.2
(diluted)		4.15	5.06	20.7	—	0.7	0.7	6.35	—	4.3	—	0.81	—

TABLE XXII—*Continued*

Species	Date collected 1926	Conc'n by refract. %	Conc'n by drying %	Coagulation by dialysis %	Crystalloidal content %	Osmotic pressure atm.	Conductivity at 25° C. K×10⁶	Hydrogen ion conc'n pH	Gold number	Bound water %	Ratio colloids: crystalloids	Bound water per gm. colloid in gm.	Per cent colloid / gold number
T. turgidum var. Alaska	June 30*												
Fresh juice		14.11	9.91	—	4.8	9.7	15.0	5.83	0.04	5.7	1.04	1.02	124.6
Dialysed 40 hr.		3.57	5.06	50.4	—	0.9	1.1	5.86	0.18	2.8	—	0.53	28.1
	July 20†												
Fresh juice		18.43	18.45	—	11.5	17.4	15.6	6.14	0.11	9.1	0.60	1.07	62.9
Dialysed 48 hr.		6.72	6.92	2.8	—	—	—	—	0.50	—	—	—	13.8
(diluted)		4.15	4.41	5.8	—	0.7	0.6	6.40	—	3.6	—	0.78	—
T. durum var. Kubanka	June 24*												
Fresh juice		12.12	11.74	—	5.7	11.0	15.2	6.08	0.08	4.8	1.05	0.71	75.1
Dialysed 40 hr.		4.65	6.01	25.0	—	1.0	1.0	6.20	0.19	3.3	—	0.52	31.6
	July 14†												
Fresh juice		15.35	15.08	—	8.1	16.5	19.8	5.91	0.10	9.6	0.85	1.18	69.5
Dialysed 48 hr.		4.65	6.95	34.3	—	—	—	—	0.51	—	—	—	13.6
(diluted)		—	5.01	—	—	0.6	1.0	6.12	—	2.3	—	0.43	—
T. polonicum var. White Polish	June 15*												
Fresh juice		9.71	—	—	—	9.0	12.7	6.22	—	5.0	—	—	—
	June 23*												
Fresh juice		11.54	11.20	—	6.1	10.7	14.5	5.96	0.14	3.4	0.85	0.59	36.8
Dialysed 40 hr.		4.15	5.15	21.7	—	0.9	0.9	6.15	0.18	1.7	—	0.31	28.6
	July 15†												
Fresh juice		14.19	14.08	—	8.1	15.7	20.4	5.89	0.08	5.5	0.74	0.79	75.1
Dialysed 48 hr.		5.06	6.01	16.5	—	—	—	—	0.38	—	—	—	15.8
(diluted)		—	4.89	—	—	0.5	0.7	6.13	—	1.0	—	0.20	—
T. dicoccum var. Common emmer	June 14*												
Fresh juice		12.1	—	—	—	10.9	13.0	6.34	—	5.5	—	—	—
	June 15*												
Fresh juice		9.30	—	—	—	8.9	14.4	6.11	—	5.2	—	—	—
	June 21*												
Fresh juice		13.78	13.26	—	5.4	10.7	11.7	6.05	0.20	6.6	1.46	0.73	39.4
Dialysed 40 hr.		9.71	7.88	0.0	—	0.8	1.2	6.12	0.21	4.9	—	0.57	37.5
	July 19†												
Fresh juice		16.18	13.78	—	5.7	14.1	16.9	6.44	0.07	10.2	1.40	1.09	114.9
Dialysed 48 hr.		6.64	8.04	29.6	—	—	—	—	0.36	—	—	—	22.3
(diluted)		4.15	5.07	30.3	—	0.6	0.8	6.25	—	1.7	—	0.32	—
T. monococcum, Einkorn	June 28*												
Fresh juice		12.45	12.07	—	5.5	11.9	17.0	6.16	0.12	6.4	1.21	0.85	55.1
Dialysed 40 hr.		4.56	6.61	33.1	—	1.0	1.1	6.20	0.12	4.1	—	0.58	55.1
	July 19†												
Fresh juice		14.36	13.80	—	7.4	13.2	16.4	6.23	0.11	5.3	0.87	0.71	58.4
Dialysed 48 hr.		4.15	6.42	37.9	—	1.4	1.2	6.05	0.53	2.3	—	0.34	12.1

*From plots seeded May 6.
†From plots seeded June 14.

TABLE XXIII

PROPERTIES OF FRESH AND DIALYSED PRESS JUICE OF GRASSES DURING SEASON OF 1926

Species	Date collected 1926	Conc'n by refract. %	Conc'n by drying %	Coagulation by dialysis %	Crystalloidal content %	Osmotic pressure atm.	Conductivity at 25° C. K×10⁶	Hydrogen ion conc'n pH	Gold number	Bound water %	Ratio colloids: crystalloids	Bound water per gm. colloid in gm.	Per cent colloid / gold number
Phleum pratense	May 26												
Fresh juice		10.87	—	—	—	16.2	18.5	5.73	—	6.4	—	—	—
	July 13												
Fresh juice		11.79	11.90	—	10.5	15.7	18.8	5.80	0.09	5.6	0.13	3.54	15.5
Dialysed 48 hr.		1.24	1.40	10.6	—	0.4	0.5	5.61	0.48	0.4	—	0.26	2.9
Agropyron tenerum	May 26												
Fresh juice		17.51	—	—	—	21.2	16.9	5.39	—	10.3	—	—	—
	July 13												
Fresh juice		16.35	16.70	—	6.8	16.1	17.3	5.79	0.09	10.2	1.27	0.85	110.2
Dialysed 48 hr.		6.14	9.92	36.8	—	1.9	3.5	5.63	0.53	7.0	—	0.63	18.7
Agropyron smithii	July 8												
Fresh juice		16.43	16.39	—	9.2	21.1	24.5	5.71	0.19	8.3	0.77	0.97	37.7
Dialysed 48 hrs.		4.90	7.16	31.7	—	0.9	1.8	5.89	0.19	4.9	—	0.63	37.7
**Bouteloua gracilis*	July 27												
Fresh juice		40.57	38.90	—	35.8	59.9	56.7	5.74	0.04	28.6	0.09	5.67	77.0
Dialysed 48 hr.		3.66	3.08	(0.0)	—	0.5	1.1	5.46	0.15	5.1	—	1.61	20.5
†Stipa comata	July 28												
Fresh juice		19.07	17.79	—	15.3	27.7	32.1	5.92	0.06	9.6	0.16	3.16	41.5
Dialysed 48 hr.		2.57	2.49	(3.7)	—	0.2	0.4	5.39	0.27	5.0	—	1.96	9.2
Poa pratensis	July 29												
Fresh juice		12.53	13.01	—	11.4	16.4	17.9	6.02	0.12	5.9	0.14	3.14	13.7
Dialysed 48 hr.		1.33	1.64	15.8	—	0.2	0.2	5.82	0.21	2.6	—	1.58	7.8
Bromus inermis	July 29												
Fresh juice		14.61	14.60	—	11.6	18.7	23.0	5.30	0.22	10.0	0.26	2.81	13.8
Dialysed 48 hr.		2.24	3.04	26.4	—	0.2	0.5	5.38	2.65	4.0	—	1.27	1.1
‡Calamovilfa longifolia	Aug. 3												
Fresh juice		12.59	12.81	—	11.8	17.8	20.5	5.84	0.09	3.3	0.09	2.81	11.4
Dialysed 48 hr.		1.22	1.03	(0.0)	—	0.2	0.2	5.52	0.16	3.3	—	3.19	6.4
Calamagrostis canadensis	Aug. 4												
Fresh juice		11.54	11.97	—	9.5	13.4	18.0	5.49	0.08	3.2	0.26	1.16	30.5
Dialysed 48 hr.		1.74	2.44	26.0	—	0.2	0.3	5.32	1.00	1.9	—	0.77	2.4
Fluminea festucacea	Aug. 10												
Fresh juice		15.77	15.19	—	10.7	19.1	15.7	5.65	0.24	5.8	0.44	1.11	18.5
Dialysed 48 hr.		2.41	4.45	47.8	—	0.2	0.5	5.98	1.45	2.7	—	0.59	3.1
Panicularia grandis	Aug. 12												
Fresh juice		11.04	10.67	—	8.1	15.5	16.0	5.27	0.13	2.2	0.32	0.76	20.1
Dialysed 48 hr.		1.74	2.61	35.6	—	0.1	0.4	5.64	0.33	1.1	—	0.41	7.9
Beckmannia erucaeformis	Aug. 13												
Fresh juice		9.63	9.63	—	7.0	12.2	17.7	5.75	0.07	1.5	0.37	0.51	37.6
Dialysed 48 hr.		1.66	2.63	36.9	—	0.2	0.4	5.67	0.38	1.1	—	0.43	6.9

**Leaf tissue collected in dry condition, and water added to extract juice. Concentration, bound water, osmotic pressure and conductivity corrected by multiplying by dilution factor: 4.405.*
†Leaf tissue collected in dry condition, and water added to extract juice. Concentration, bound water, osmotic pressure and conductivity corrected by multiplying by dilution factor: 3.892.
‡Leaf tissue collected in dry condition, and water added to extract juice. Concentration, bound water, osmotic pressure and conductivity corrected by multiplying by dilution factor: 2.445.

however, reduced the number of collections it was possible to make of the wheats and cultivated grasses. A larger quantity of juice was of course required, and because the wheat leaves of the last collection, though young, were rather dry, the dialysed juice had to be diluted to obtain sufficient to complete all the determinations satisfactorily. For the same reason, the grass leaves collected from the hillside, where habitat conditions were drier than the previous season, had to have water added before an adequate quantity of juice could be extracted.

TABLE XXIV

EFFECT OF DILUTION ON PROPERTIES OF DIALYSED PRESS JUICE

Variety	Date collected 1926	Conc'n by refract. %	Conc'n by drying %	Apparent degree of coagulation %	Osmotic pressure atm.	Conductivity at 25° C. $K \times 10^6$	Hydrogen ion conc'n pH	Bound water per gm. colloid in gm.
Hybrid 143 wheat	July 20							
Dialysed 48 hr.		6.97	8.14	16.5	—	—	—	—
Diluted		4.15	4.98	18.7	—	—	—	—
Marquis wheat	July 21							
Dialysed 48 hr.		8.22	9.22	13.3	—	—	—	—
Diluted		4.15	4.70	14.2	—	—	—	—
Caesium wheat	July 21							
Dialysed 48 hr.		6.39	7.56	18.4	—	—	—	—
Diluted		4.15	4.85	17.3	—	—	—	—
White spelt	July 20							
Dialysed 48 hr.		7.39	8.89	19.6	—	—	—	—
Diluted		4.15	5.06	20.7	—	—	—	—
Alaska wheat	July 20							
Dialysed 48 hr.		6.72	6.92	2.8	—	—	—	—
Diluted		4.15	4.41	5.8	—	—	—	—
Common emmer	July 19							
Dialysed 48 hr.		6.64	8.04	29.6	—	—	—	—
Diluted		4.15	5.07	30.3	—	—	—	—
Western rye grass	July 13							
Dialysed 48 hr.		6.14	9.92	36.8	1.93	3.51	5.63	0.63
Diluted		1.24	2.15	41.1	0.72	1.20	5.85	0.58
Western wheat grass	July 8							
Dialysed 48 hr.		4.90	7.16	31.7	0.89	1.77	5.89	0.63
Diluted		3.24	4.04	20.0	0.60	1.25	5.96	0.86
Awnless brome grass	July 29							
Dialysed 48 hr.		2.24	3.04	26.4	0.24	0.47	5.38	1.27
Diluted		1.35	1.74	23.7	0.14	0.34	5.61	1.47
Prickle fescue grass	Aug. 10							
Dialysed 48 hrs.		2.41	4.45	47.8	0.23	0.46	5.98	0.59
Diluted		1.24	2.09	42.9	0.16	—	6.03	0.65

To obtain some information as to the effect of dilution on dialysed juice, complete series of determinations were run on the juice both as dialysed and after further dilution, in the case of a few grasses of which an abundance of

leaves in succulent condition was readily available. The results are given in Table XXIV, together with data brought forward from Table XXII, on the concentration of dialysed wheat-juice samples with and without dilution. The concentration was determined by both refractometer (with 17% correction) and oven-drying, and, as discussed in Section V, from the change in the relative magnitudes of these two readings may be calculated the degree of coagulation. The coagulation of course takes place mainly during dialysis, a process which, so far as crystalloids are concerned, amounts to infinite dilution. It is therefore not surprising to find very little additional coagulation on further dilution. In fact, in the grasses, in three out of four cases shown in Table XXIV, dilution of the dialysed juice leads to dispersion and increased hydration (bound water per gram) of colloids. Such a result would be expected from a consideration of the relation between concentration and hydration brought out earlier (Table II). The other properties shown in Table XXIV, *viz.*, osmotic pressure, conductivity, and hydrogen ion concentration, behave normally on dilution. The changes in the first two have probably little significance, but the slight movement of the last towards neutrality no doubt partly offsets the tendency to increased hydration of colloids with dilution. While therefore, from a comparative standpoint, the dilution of the dialysed juice of certain of the wheat samples is objectionable, it does not entirely invalidate the results. It should be noted, moreover, that the classification made later of the various species according to drought resistance, is based on the properties of the fresh juice.

In Tables XXII and XXIII, the concentration of the dialysed juice (undiluted), as determined by oven-drying, is taken as the colloidal content, and the difference between this and the concentration of the fresh juice is listed as the crystalloidal content. The ratio of colloids to crystalloids given later in the tables varies widely, and possibly characteristically, in different species, but does not form a basis on which the species can be logically classified in order of drought resistance. This ratio must of course be affected by environmental conditions, such as concentration of the soil solution, and by metabolic balance, as in starch-sugar equilibrium. These factors would be operative with the grasses more than with the wheats, since the latter were all growing under closely similar conditions.

The stability of the colloids, as reflected in degree of coagulation on dialysis, is apparently not characteristic, since it varies widely in different collections of the same species. It is probably influenced strongly by physiological state at the time of collection. The same is no doubt true of the gold number, which must depend largely on the temporary state of dispersion of the colloids. It increases on dialysis, when coagulation takes place.

The bound water per gram of colloid in the fresh juice, as reported in the tables, takes no account of the hydration of crystalloids, and the reduction on dialysis may be partly due to the removal of other hydrated substances. This seems the more likely since the average drop is greater in the grasses, which average lower than the wheats in ratio of colloids to crystalloids. The removal of salts by dialysis would of course be expected to result in precipitation of the

globulins, but the extremely variable amounts of coagulation indicated in Tables XXII and XXIII, even in different collections of the same species, do not afford grounds for believing that this reaction was confined to the globulin fraction. While the coagulation during dialysis would seem in many cases sufficient to account for the decreased hydration, it has been seen earlier (Section V) that these two phenomena are not always logically connected, and in Table XXII a few of the wheats show increased hydration associated with at least a moderate degree of coagulation. There is of course the possibility of a shift in the isoelectric point on the removal of electrolytes, which would probably affect hydration.

The osmotic pressure and conductivity of the dialysed juices are usually within the ranges found in prepared sols of corresponding concentration (Table II), and indicate satisfactory dialysis.

The coefficients of correlation between various press-juice properties found in 1926 are given in Table XXV. Those for concentration, osmotic pressure, and bound water of fresh juice will be discussed later in summarizing these coefficients for all three seasons. The other coefficients, however, apply only to the work of 1926.

Colloidal content and bound water show less apparent relation than might be expected. The coefficient is statistically significant only in the case of the fresh juice of the wheats. When the colloidal content is multiplied by the reciprocal of the gold number (the reciprocal is used because the gold number is inversely proportional to protective quality), the correlation is improved. The gold number itself, however, shows significant correlation with bound water per gram of colloid again only in the fresh wheat juices, the coefficient in this case being, as expected, negative.

The hydrogen ion concentration of all samples was on the acid side of neutrality and, probably, of the isoelectric point of the colloids (Fig. 12, Section V). It would therefore be expected to find a negative correlation between pH and bound water per gram of colloid. This, however, occurred only in the dialysed juice, and in the wheats the coefficient is too small to be significant. The grass juices were appreciably more acid than the wheat juices, and on dialysis the reaction of the former, unlike that of the latter, shifted in most cases in the acid direction. This may account for the differences in the coefficients of the two classes of plants. The range of pH values in both crops was small, perhaps too small to have much effect on hydration, while the differences in hydration were quite large. With such unequal combinations the magnitude and sign of the correlation coefficients may perhaps be largely fortuitous.

The whole set of correlation coefficients depending upon the properties of the dialysed colloids (No. 4 to 12 in Table XXV) is perhaps of doubtful validity, since it is based on figures which in most cases show large variability, while the number of cases, especially in the grasses, is rather small to be effectively treated in this way. As an example, the actual numerical relation between gold number and bound water per gram of colloid is given at the bottom of the table. Gold number nearly always increases with dialysis and bound

TABLE XXV

COEFFICIENTS OF CORRELATION BETWEEN PRESS-JUICE PROPERTIES OF WHEATS AND GRASSES DURING SEASON OF 1926

Properties	Wheats		Grasses	
	No. of cases	Coeff. of correlation	No. of cases	Coeff. of correlation
1. Concentration and osmotic pressure (fresh juice)	24	0.93±0.02	14	0.98±0.01
2. Concentration and bound water (fresh juice)	24	0.60±0.09	14	0.97±0.01
3. Osmotic pressure and bound water (fresh juice)	24	0.62±0.08	14	0.94±0.02
4. Colloidal content and bound water (fresh juice)	19	0.49±0.12	12	0.19±0.19
5. Do. (dialysed juice)	19	0.35±0.14	12	0.34±0.17
6. (Colloidal content+gold number) and bound water (fresh juice)	19	0.40±0.13	12	0.56±0.13
7. Do. (dialysed juice)	19	0.44±0.12	12	0.43±0.16
8. Gold number and bound water per gram colloid (fresh juice)	19	−0.42±0.13	12	−0.37±0.17
9. Do. (dialysed juice)	19	0.08±0.15	12	−0.12±0.19
10. Do. (all samples)	38	−0.31±0.10	24	−0.26±0.13
11. pH and bound water per gram colloid (fresh juice)	19	0.69±0.08	12	0.29±0.18
12. Do. (dialysed juice)	19	−0.26±0.14	12	−0.58±0.13

NUMERICAL RELATION BETWEEN GOLD NUMBER AND BOUND WATER PER GRAM COLLOID

Properties	Mean		Mean difference	Odds
	Fresh	Dialysed		
Wheat				
Gold number*	0.11	0.31	−0.20	9999.0:1
Bound water per gram colloid†	0.75	0.51	0.24	1999.2:1
Grasses				
Gold number‡	0.12	0.65	−0.53	83.2:1
Bound water per gram colloid§	2.21	1.15	1.06	89.3:1

*One out of 19 cases showed no increase on dialysis.
†Five out of 19 cases showed slight increases on dialysis.
‡One out of 12 cases showed no increase on dialysis.
§One out of 12 cases showed increase on dialysis.

water per gram of colloid usually decreases (both being related to state of dispersion and affected by coagulation during dialysis). The mean differences before and after dialysis are large, and the odds (by Student's method) indicate that the differences are very significant. Such a set of figures would lead to the expectation of a strong negative correlation between gold number and

bound water per gram, but this is not shown by the coefficients, for the reasons given above.

It is interesting to note that the mean gold numbers of the colloids in the fresh juice of both wheats and grasses are practically identical. The much higher hydration in the grasses is probably due in part to the lower mean concentration of colloids, 3.5% as compared with 7% in the wheats.

This study of the colloidal content of plant juices, and various factors affecting or reflecting its state of dispersion and hydration, indicates the difficulty of elucidating or assigning quantitative values to particular relations. The very labile colloids of plant cells are not easily isolated without an indefinite amount of change in state, and relationships which the weight of several experiments show must exist are hard to demonstrate quantitatively. The effect of one factor is usually masked by simultaneous variation in several others, and the variations are not readily controlled or measured. Nevertheless, the results give reason to believe that as methods of controlling these factors are developed, protoplasmic colloids will be found susceptible of the same quantitative studies as have been carried out with prepared colloids.

In regard to the practical problem we find that of all the properties listed in Tables XXII and XXIII, only per cent bound water makes it possible to classify logically both wheats and grasses with respect to drought resistance. The grasses, but not the wheats, fall into a fairly similar classification on the basis of concentration and osmotic pressure of juice. The classification of all crops, however, works out best if we use the total bound water of the fresh juice. It appears that at least until methods of dialysis have been developed which protect the colloids from alteration during the process, the simpler measure of bound water in fresh juice remains also a more satisfactory ecological index.

Discussion

The principal issues of the results for the three seasons of 1924-25-26 may now be considered. The greatest practical interest centres around the classification of the wheats and grasses on the basis of bound water in the press juice. This is given in Tables XXVI and XXVII. The values there are brought forward from the tables for 1925-26, averaged where series of collections were made, except that the two collections of common emmer made on June 14-15, 1926 (Table XXII) are treated as one to make this variety comparable with the others, and the first collections of timothy and western rye grass in 1926 (Table XXIII) are excluded for the same reason.

With cultivated crops such as wheat it is more difficult to get accurate information as to relative drought resistance, since one cannot judge directly from the habitats as in the wild grasses. Nevertheless, the classification in Table XXVI is logical so far as can be judged from common knowledge and experience. Emmer and durum wheat are both grown more successfully than common wheat under dry-farming conditions. Caesium, the common wheat variety which appears in third place, was introduced from Russia because of its reputed high drought resistance. It turned out to have inferior baking

quality as judged by our standards, but should be valuable to plant breeders as a source of the drought-resistant factor. Spelt has a reputation inferior to that of emmer as a feed wheat, because of the greater resistance of the latter to both drought and disease. The club wheats, of which Hybrid No. 143 is an example, are preferred in the dry intermountain regions of the western United States because of various characters which include greater drought resistance than most common wheats. Marquis, the most widely grown variety of common wheat on this continent, in spite of its excellent qualities is not satisfactorily drought resistant, and plant breeders are now attempting to improve it in this respect. The other varieties listed in Table XXVI are not commonly grown, but such information as is available indicates that they fall into about the right places.

TABLE XXVI

WHEAT SPECIES AND VARIETIES ARRANGED IN ORDER OF DROUGHT RESISTANCE AS INDICATED BY AVERAGE BOUND WATER IN SEASONS OF 1925 AND 1926

Species	1925		1926		Average	
	Osmotic pressure atm.	Bound water %	Osmotic pressure atm.	Bound water %	Osmotic pressure atm.	Bound water %
1. *Triticum dicoccum* var. Common emmer	14.3	7.10	11.6	7.39	12.9	7.2
2. *T. durum* var. Kubanka	13.9	6.73	13.8	7.23	13.8	7.0
3. *T. turgidum* var. Alaska	13.3	6.38	13.6	7.43	13.4	6.9
4. *T. vulgare* var. Caesium	13.9	6.67	14.5	6.43	14.2	6.5
5. *T. compactum* var. Hybrid 143	12.8	5.53	13.3	5.98	13.0	5.8
6. *T. spelta* var. White spelt	14.2	5.72	15.3	5.58	14.7	5.6
7. *T. monococcum*, Einkorn	12.7	5.42	12.6	5.84	12.6	5.6
8. *T. polonicum* var. White Polish	13.0	4.20	11.8	4.66	12.4	4.4
9. *T. vulgare* var. Marquis	14.6	3.87	12.5	4.30	13.5	4.1

Coefficients of correlation

	n	*r*
Osmotic pressure, 1925, and osmotic pressure, 1926	9	0.20 ± 0.22
Bound water, 1925, and bound water, 1926	9	0.96 ± 0.02

A logical development of these experiments would be to grow the varieties under a series of controlled moisture conditions, from optimum to minimum, and observe the effect on growth, yield and juice properties.

The adaptations of the grasses are so well known as to leave no doubt of the approximate correctness of the placing given in Table XXVII. *Bouteloua gracilis* and *Stipa comata* are native and abundant in the dry plains of southern Alberta and Saskatchewan. *Agropyron cristatum* was introduced from the dry regions of Siberia and gives promise in our semi-arid districts. *Agropyron tenerum* and *Bromus inermis*, the former native and the latter introduced, are the best hay grasses in western Canada, having largely because of their drought resistance displaced *Phleum pratense*, so popular in moister parts of America but ill-adapted to dry regions. *Agropyron smithii* is native and often dominant

TABLE XXVII

GRASSES ARRANGED IN ORDER OF DROUGHT RESISTANCE AS INDICATED BY AVERAGE BOUND WATER IN SEASONS OF 1925 AND 1926

Species	1925		1926		Average	
	Osmotic pressure atm.	Bound water %	Osmotic pressure atm.	Bound water %	Osmotic pressure atm.	Bound water %
1. *Bouteloua gracilis*, Blue grama	34.9	16.73	59.9	28.59	47.4	22.7
2. *Stipa comata*, Western spear	23.2	15.14	27.7	9.58	25.4	12.4
3. *Agropyron cristatum*, Crested wheat	20.3	11.70	—	—	—	—
4. *Agropyron tenerum*, Western rye	18.7	10.49	16.1	10.18	17.4	10.3
5. *Bromus inermis*, Awnless brome	19.7	10.60	18.7	9.99	19.2	10.3
6. *Agropyron smithii*, Western wheat	17.4	7.09	21.1	8.27	19.2	7.7
7. *Poa pratensis*, Kentucky blue	13.4	4.66	16.4	5.92	14.9	5.3
8. *Calamovilfa longifolia*, Sand grass	13.2	6.91	17.8	3.32	15.5	5.1
9. *Fluminea festucacea*, Prickle fescue	13.4	4.29	19.1	5.85	16.2	5.1
10. *Phleum pratense*, Timothy	15.7	3.43	15.7	5.62	15.7	4.5
11. *Calamagrostis canadensis*, Blue joint	11.9	3.71	13.4	3.22	12.6	3.5
12. *Panicularia grandis*, Tall manna	9.7	4.40	15.5	2.22	12.6	3.3
13. *Beckmannia erucaeformis*, Slough grass	11.3	3.09	12.2	1.48	11.7	2.3

Coefficients of correlation

	n	r
Osmotic pressure, 1925, and osmotic pressure, 1926	12	0.92 ± 0.03
Bound water, 1925, and bound water, 1926	12	0.82 ± 0.06

in the dry plains, and if it had been collected from its usual habitat rather than from a cultivated plot (seeded thinly in May, 1925) it might have occupied a higher position in the list. The remaining grasses represent a lower order of drought resistance. The last two are found only in sloughs and wet places, while *Calamagrostis canadensis* forms the chief constituent of many valuable, low-lying natural meadows around the margins of some Alberta lakes.

The bound-water values for given wheat varieties in the two seasons are close enough to suggest that they are characteristic. Such changes as occur in the order of the varieties in the two years are caused by small differences. The coefficient of correlation between bound water, 1925, and bound water, 1926, is 0.96 ± 0.02. The grasses show a little more variability in this respect, perhaps because the values are based largely on one collection per species. The correlation in bound water for the two seasons is still high, the coefficient being 0.82 ± 0.06.

Osmotic pressure in the wheat species afforded no indication of ecological adaptation. This property, unlike bound water, is probably not characteristic, but controlled mainly by habitat conditions. The wheats were grown on very uniform field plots, and their osmotic pressures varied only within narrow limits. Such variations as existed are evidently not characteristic of the species, since there is no correlation between the values for 1925 and 1926. The same observations apply in a less marked degree to the three grasses which were grown side by side in field plots. Comparing the osmotic values of 1926 with 1925, *Agropyron tenerum* decreased, *A. smithii* increased, *Phleum pratense* showed no change, and all values for both seasons fell within a comparatively narrow range. Taking the whole series of grasses, however, they were collected from very distinctive habitats, the same both seasons, and the osmotic values for the two seasons are highly correlated. They also show a considerable relation to drought resistance, though less marked or complete than that shown by bound water. It is difficult to avoid the conclusion that the osmotic values are mainly the result of environment, and that their relation to drought resistance arises merely from the fact that the leaves were collected in typical habitats.

That the same argument cannot be applied to the bound-water values is indicated by the distinctive series found in the wheats grown under uniform conditions, and by a consideration of certain selected groups of grasses. It has been mentioned that *Agropyron tenerum*, *A. smithii* and *Phleum pratense* were grown side by side in uniformly prepared field plots. Their average bound-water values were 10.3, 7.7, and 4.5%, respectively, showing a percentage spread vastly greater than in their osmotic values, and indicative of their drought adaptations. On the other hand, *Poa pratensis* and *Fluminea festucacea*, collected respectively from a dry part of the University campus and from the margin of a slough, had 5.3 and 5.1% bound water, suggesting that in this property mesophytic grasses tend to fall within a restricted range regardless of habitat.

It is not intended to discount the importance of osmotic pressure in the water relations of plants. Undoubtedly, osmotic and imbibition pressure are inseparably connected in this regard, a point which will be touched again later. Also, it is quite probable that osmotic pressure varies characteristically in certain ecological types; we note that it runs on the average appreciably higher in the grasses than in the wheats, even when the two are grown in similar field plots. Nor is it suggested that bound-water content is wholly unaffected by environment; instances to the contrary could easily be pointed out. But if, as seems probable, it is even a moderately stable and characteristic property of individual species, it becomes valuable as a measure of inherent adaptation.

The maximal values for bound water are of interest. They are found in the two xerophytic grasses, *Bouteloua gracilis* and *Stipa comata*. If we disregard the values for these species found in 1926, which are open to criticism inasmuch as water was added to the leaves in extracting the fluids, we find that in 1925 they were 16.7 and 15.1%. With this may be compared the value for *Opuntia*

polyacantha, a native cactus collected in southern Alberta in July, 1925, which, with a juice concentration of only 5.1% (the lowest observed in any plant studied) and an osmotic pressure of 7.6 atm., had a bound-water content of 16.6%. Undoubtedly these three were the most drought-resistant plants studied.

The colloidal content and bound water of course affect the viscosity of the juice. The juice of timothy, for example, was highly fluid, while that of western rye grass was very viscous and upon standing a couple of hours set to a gel (Plate IV-B). The latter property, while unique among the plants included in this investigation, is not necessarily related to drought resistance as it has since been observed by one of the authors in the juice of *Typha*, the cat-tail. Presumably it is the result of a pectic reaction. Maximum viscosity, however, was found in the cactus juice, which had to be diluted to about 1.3% concentration before it could be handled satisfactorily in making the various determinations.

Since of the properties investigated, the bound water of the fresh juice seems the best index of drought resistance, with concentration and osmotic pressure also showing considerable relation to moisture adaptations in the grasses, it is of interest to examine the correlations between these three properties. The coefficients are summarized in Table XXVIII.

It may be noted first that the correlations are higher than those reported by Gortner and Rude (18), especially between concentration and bound water, and between osmotic pressure and bound water. This may be partly because the plants included in the correlation tables were restricted to a narrower range of relationship. An example of the sort of error which may result from wide grouping was pointed out in the discussion of the 1924 experiments.

TABLE XXVIII

SUMMARY OF CORRELATIONS BETWEEN CONCENTRATION, OSMOTIC PRESSURE AND BOUND WATER OF FRESH PRESS JUICE, FOR 1924-25-26

Properties	1924	1925	1926
	r	*r*	*r*
Wheats			
Concentration and osmotic pressure	0.88±0.03	0.81±0.03	0.93±0.02
Concentration and bound water	0.65±0.09	0.44±0.08	0.60±0.09
Osmotic pressure and bound water	0.17±0.16	0.43±0.08	0.62±0.08
Grasses			
Concentration and osmotic pressure	—	0.78±0.05	0.98±0.01
Concentration and bound water	—	0.78±0.05	0.97±0.01
Osmotic pressure and bound water	—	0.79±0.05	0.94±0.02

It was also pointed out, in discussing the higher correlation of concentration and osmotic pressure as compared with concentration and bound water, in the wheats, that the former relationship is essentially linear while the latter is likely to be curved. The fact that both correlations are equal in the grasses may be partly due to the lower concentration of colloids. The average colloidal

content of the wheats in 1926 was 7.0% as compared with 3.5% in the grasses. Reference to the bound-water curves obtained with prepared sols (Fig. 3) will show in most cases at concentrations corresponding to those of the grasses a relationship much more nearly linear.

Since osmotic pressure follows concentration directly, it will also be related to bound water to the extent the latter is related to concentration. This is evident in both wheats and grasses. The high correlation in the grasses corresponds also with the observation that the species fall into appreciably the same order on the basis of either property. The significance of the two classifications has already been discussed.

Walter (54) states that the water content of the plasma stands in direct relation to the osmotic value: the absorptive power of the plasma, depending on the state of swelling, is always equal to the absorptive power of the cell contents. While this seems a self-evident proposition, it may be misleading unless carefully circumscribed. It must not be supposed that a certain gel will always swell to the same extent in a series of solutions of equal osmotic concentration. The composition of the solution and especially its hydrogen ion concentration may change profoundly the point at which equilibrium is reached. Again, the composition and state of dispersion of the colloid have important effects. That all of these factors are operative in plant-cell colloids is abundantly shown in these investigations. It will be remembered also that Gortner and Rude (18) found no significant correlation between osmotic pressure and bound water, and in the wheat species reported above it was far from perfect. It is not implied, of course, that bound water and imbibition pressure are identical, but they are closely related. However, with due recognition of factors which may lead to shifting of equilibria, and which must be constantly operative in physiologically active plant cells, we may assume that the movement is always towards an equalizing of osmotic and imbibition pressure.

In a transpiring plant there must be a pressure gradient through the plasma to the outside of the cell wall. As water vapor diffuses from the intercellular spaces into the external atmosphere, the vapor pressure in these spaces is lowered and water leaves the film on the surrounding walls to re-establish equilibrium between the vapor and the liquid phases. If the rate of evaporation exceeds that of absorption, water leaving the cell walls will not be fully replaced, and the concentration of the cell fluids will be increased. To maintain equilibrium the water film will recede into the pores of the cell walls, the surface force increasing with the curvature of the menisci, until it balances the imbibition and osmotic pressure of the cell fluids. The logarithmic nature of an imbibition curve means that in a cell containing a high concentration of colloids the pressure goes up very rapidly, and the rate of outward diffusion must be slower than in a cell lacking such protection. What difference, if any, an abundance of hydrophilic colloids in the cells may make in the final moisture content of the tissues, we have not as yet experimental evidence to show.

A gel state, as pointed out in reviewing the literature, seems characteristic of the cell contents in highly resistant organisms. In view of the very general

association found between hydrophilic colloids and drought resistance, it seems not unlikely that these substances besides retarding the rate of water abstraction and giving the cells time to adjust their physiological condition, may also preserve the living organization of the cell protoplasm and confer upon the plant the wilt endurance which Maximov has emphasized as the prime feature of drought resistance.

Properties of Leaf-tissue Fluids of Two Poplar Species in Relation to Frost Resistance and Leaf Fall

The contrasting behavior of two poplar species on the campus of the University of Alberta, with reference to resistance to injury by fall frosts and date of leaf fall, has been observed for several years. *Populus certinensis* retains its leaves in a healthy condition for a considerable period longer than *P. petrovski*. Those of the latter species, if they have not already fallen, are usually despatched by the first substantial frost. In the autumn of 1926, an opportunity came to examine their leaf-tissue fluids, and the results showed the expected relationship between juice properties and ecological adaptation.

The analogy between drought and frost resistance has already been mentioned, water being abstracted from the cell walls in the one case by evaporation and in the other by crystallization. It may also be pointed out that maturation of tissues is essentially a process of dehydration, and that any factor which delays dehydration will also delay maturation. Thus it is not surprising to find a species rich in colloids maturing and shedding its leaves later, and also resisting better the frosts to which its later maturity may expose it.

The history of the trees in question, in the fall of 1926, was normal. On the night of September 23-24, the temperature dropped to 18° F. (14° of frost), after which the leaves of *P. petrovski* were found to be severely damaged, while those of *P. certinensis* showed no injury except at the terminals of the topmost branches. The leaves had practically all dropped from *P. petrovski* by October 5, while this did not happen in *P. certinensis* until October 16.

Leaves from the two species, growing side by side (Plate I-B), were collected August 16, while they were in full vegetation, and again September 20, when still green but approaching maturity. The selection of the latter date turned out to be exceedingly fortunate, as a heavy frost came soon afterwards. The properties of the expressed fluids at the two dates of collection are given in Table XXIX.

The concentration of the fresh juice did not differ appreciably on August 16, but by September 20 *P. petrovski* had fallen off considerably in this respect, while *P. certinensis* had maintained its original level. The latter had, at the first date nearly double and, at the second date, more than double the absolute concentration of colloids found in the former, while the differences in ratio of colloids to crystalloids were still greater.

There is one important difference between the two species in osmotic pressure and conductivity of the fresh juice, *viz.*, the relation between the comparative values for these two properties indicates that *P. certinensis* had more of its crystalloidal content in the form of non-electrolytes. This is particularly true

TABLE XXIX

PROPERTIES OF FRESH AND DIALYSED PRESS JUICE OF LEAVES OF TWO POPLAR SPECIES IN RELATION TO FROST RESISTANCE AND LEAF FALL

Species	Date collected 1926	Conc'n by drying %	Crystalloidal content %	Osmotic pressure atm.	Conductivity at 25° C. K×10⁶	Hydrogen ion conc'n pH	Gold number	Bound water %	Ratio colloids: crystalloids	Bound water per gm. colloid in gm.	Per cent colloid / gold number
*P. certinensis**	Aug. 16										
Fresh juice		20.9	12.4	17.9	11.4	5.58	0.38	12.8	0.69	1.19	22.5
Dialysed 86 hr.		8.5		1.5	1.4	5.51	2.10	9.0		0.96	4.1
P. petrovski†	Aug. 16										
Fresh juice		19.7	14.8	18.7	14.4	5.66	0.18	11.4	0.33	1.85	27.4
Dialysed 86 hr.		4.9		0.6	0.8	5.72	0.76	4.1		0.79	6.5
*P. certinensis**	Sept. 20										
Fresh juice		20.6	13.1	19.3	11.0	5.40	0.27	12.0	0.57	1.27	27.7
Dialysed 86 hr.		7.5		1.4	1.5	5.51	1.23	5.9		0.73	6.1
P. petrovski†	Sept. 20										
Fresh juice		16.0	12.9	15.8	13.1	5.29	0.14	9.3	0.24	2.52	22.1
Dialysed 86 hr.		3.1		0.8	1.5	5.57	0.39	2.7		0.84	7.9

**Approximate date of leaf fall, Oct. 16. No apparent injury to leaves by 14° F. frost, Sept. 23-24.*

†*Approximate date of leaf fall, Oct. 5. Leaves killed by 14° F. frost, Sept. 23-24.*

of the second collection, when sugars would be especially valuable as a protection against frost-precipitation of the protoplasmic proteins.

In hydrogen ion concentration of the fresh juice the two species are essentially alike, but between the two collections there is an appreciable shift in the acid direction. This is associated with an increase in bound water per gram of colloid, and probably with increased dispersion as indicated by a decreased gold number.

P. certinensis had a greater content of bound water than *P. petrovski*, though the differences in the fresh juice, especially in the first collection, were not as large as the differences in colloidal content; that is, bound water per gram of colloid was greater in *P. petrovski*. This is interestingly related to the gold number, which indicates a higher protective quality in the colloids of *P. petrovski*. It has frequently been observed that in plants low in colloids the gold number quality is better. Whether this follows merely from greater dispersion and hydration as a result of lower concentration, or indicates a real difference between strictly protoplasmic colloids and those developed in response to physiological conditions, cannot be said with certainty. Probably both explanations are true in part. In the present case, the total bound-water content of the fresh juice is more nearly proportional to the values obtained when the per cent colloid is multiplied by the reciprocal of the gold number (that is, when both quantity and quality of colloid are considered) than it is to the per cent colloid alone.

By this experiment, the general conclusions as to the relation between juice properties and drought resistance are logically extended and found to apply to related ecological phenomena.

Summary

The concentration, osmotic pressure and bound water of leaf press-juice of the wheat species were determined at intervals during the seasons 1924-25-26. The same properties were determined on other cereals and a few miscellaneous crops in 1924, and on a series of cultivated and wild grasses in 1925-26. In 1926 the colloids of both wheats and grasses were separated by dialysis, and various properties of the dialysed juice determined.

The wheat juices contained on the average twice as much colloidal material as the grasses and, with the same gold number, the average hydration of the latter was greater. Owing to partial coagulation of colloids during dialysis, the gold number increased and the hydration usually decreased. The changes on dialysis were so irregular as to obscure correlations which probably exist. It is concluded that until methods are developed which protect the colloids from alteration during dialysis, the properties of the fresh juice are more satisfactory ecological indices.

Concentration, osmotic pressure and bound water of juice all tend to increase with the progress of maturity, the last proportionately fastest. Wheat, spring rye, barley and oats fell in this order in magnitude of values for the foregoing properties.

Both grasses and wheats could be classified very satisfactorily with respect to drought resistance on the basis of the bound-water content of the fresh juice. In the grasses there was also found considerable relation between drought adaptation and concentration and osmotic pressure of juice, but it is believed that this was the result more of collecting them in typical and distinctive habitats than of characteristic differences in the species. It seems probable that bound-water content is a more stable and characteristic property than osmotic pressure. It is suggested that a high colloidal content must reduce the rate of abstraction of water from plant cells and may also confer on a plant the property of wilt endurance.

The coefficients of correlation between various juice properties, especially concentration, osmotic pressure, and bound water, are given and discussed.

Populus certinensis, which holds its leaves longer in the fall and is highly frost resistant, has a higher content of bound water, colloids, and probably also of sugars, than *P. petrovski*, of which the leaves fall earlier and are non-resistant to frost.

VII. Experiments with Plant Tissues

The investigations reported so far in this paper have dealt entirely with the properties of expressed plant-tissue fluids. A few additional experiments carried out with the tissues themselves are reported in this section.

Direct Measurement of Imbibition Pressure

An attempt was made to measure the imbibition pressure of the leaf tissue by the direct-pressure method used by Newton (37) in his investigation of frost resistance in winter wheat. The programme was not completed in this regard, only one series of experiments being carried out, in the season of 1924.

This preliminary series was done with the same type of steel press bowl, about 3 in. in diameter, used in the experiments just cited. The piston fitted loosely enough to allow the sap to pass around it, the bowl being inverted in a small metal collecting vessel which drained into a graduated cylinder. Fifty-gram samples of fresh leaves were folded in discs of strong cotton, of standard size, which had been previously moistened with sap by pressing out with another sample of leaves, in order to avoid errors due to absorption by the cotton. Pressure was applied in successive steps of 100 atm., from 100 to 600 atm., the fluid being allowed to drain for 2.5 min. with each increase in pressure. The volume of juice expressed at each pressure was noted. The experiments were carried out in triplicate for each variety.

The figures recorded in Table XXX have been restricted to the average initial and final moisture content of the leaves, and the average volume and concentration of the sap obtained at 600 atm. pressure. The actual volumes of sap were small (they have been doubled in the table to bring them to a basis uniform with that of the other figures recorded), and small differences in the actual quantities obtained in the triplicate determinations made in consequence such large percentage errors, that it seemed scarcely justifiable to construct hydration curves based on the volumes of sap obtained at various pressures from 100 to 600 atm. These data have therefore been omitted.

TABLE XXX

RESISTANCE OF LEAVES TO EXTRACTION OF SAP AT 600 ATM. PRESSURE

Species	Date collected 1924	Water in 100 gm. leaves		Volume of sap from 100 gm. leaves* cc.	Conc'n of sap by refract. %
		Initial gm.	Final gm.		
Triticum compactum var. Hybrid 143	Aug. 18	66.4	55.4	12.0	13.1
T. vulgare var. Marquis	Aug. 18	68.4	61.3	7.8	12.0
T. spelta var. White spelt	Aug. 19	70.1	50.2	21.2	8.0
T. turgidum var. Alaska	Aug. 18	71.4	54.8	17.8	9.1
T. durum var. Kubanka	Aug. 18	71.8	57.5	15.2	8.5
T. polonicum var. White Polish	Aug. 18	70.1	56.6	14.6	10.7
T. dicoccum var. Common emmer	Aug. 19	72.8	52.1	22.0	8.1
T. monococcum Einkorn	Aug. 19	76.1	52.4	25.0	6.7
Avena sativa var. Banner	Aug. 11	78.3	61.8	17.2	5.6
A. sativa var. Leader	Aug. 11	77.9	58.1	20.8	6.5
A. nuda var. Chinese hulless	Aug. 11	80.1	60.2	20.6	4.6
A. fatua Wild oats	Aug. 11	80.1	57.4	23.6	5.2
A. sterilis Red oats	Aug. 11	78.0	63.3	15.4	6.1
A. sterilis var. *ludoviciana*	Aug. 11	79.8	55.0	24.8	4.2
A. strigosa Sand oats	Aug. 11	76.6	61.4	16.0	5.8
A. brevis Short oats	Aug. 11	77.6	58.3	20.0	4.1
Hordeum vulgare var. O. A. C. No. 21	Aug. 8	78.1	51.3	28.4	7.3
H. vulgare var. Barks	Aug. 8	73.4	55.0	19.6	7.6
H. distichon var. Canadian Thorpe	Aug. 8	77.3	59.9	18.2	6.0
H. distichon var. Hannchen	Aug. 8	75.5	54.8	20.0	6.9
Secale cereale var. O. A. C. No. 62	Aug. 9	73.6	55.4	19.8	10.5
S. cereale var. Alberta winter	Aug. 9	81.2	49.6	33.4	7.3
S. cereale var. Rosen	Aug. 9	81.9	64.6	18.2	6.7
Phleum pratense Timothy	Aug. 9	80.1	56.4	24.0	1.6
Agropyron tenerum Western rye grass	Aug. 9	65.3	45.0	21.4	6.7

**By doubling volumes obtained experimentally from 50-gm. samples.*

The results do not appear to have any relation to the comparative drought resistance of the varieties. The method may hence be unsuitable to the measurement of this quality. It is to be noted, however, that the experiments were undoubtedly carried out too late in the season, all of the collections being made in August. By this time the plants in most cases were approaching maturity, and the various stages of advancement of the varieties affected their initial hydration, while the development of woody tissue no doubt opposed mechanical resistance to the effective compression of the mass of leaves and affected the final hydration under pressure. The relation of volume of juice and final hydration of the tissue to frost resistance in winter wheat, found in the earlier investigations cited, may have been due to the use of very young plants in which practically no secondary structural tissue had been laid down, and which therefore may have behaved under pressure much like a colloidal gel.

It is of interest to note the high resistance of the leaves to this pressure desiccation. For a mass of leaves to maintain 57% hydration of its dry matter under a pressure of 600 atm., as happened in the case of Rosen rye, is very striking. It is also of interest to compare the concentration of the juice obtained from entire leaves at high pressure with that obtained from ground leaves at low pressure. In Table XVII it was seen that the latter method applied to tissues from the same plots at the same time of the year resulted in concentrations which on the average were more than double those reported in Table XXX. The omission of grinding and the compacting by high pressure undoubtedly led to the screening-out of a large proportion of the solutes, especially colloidal substances.

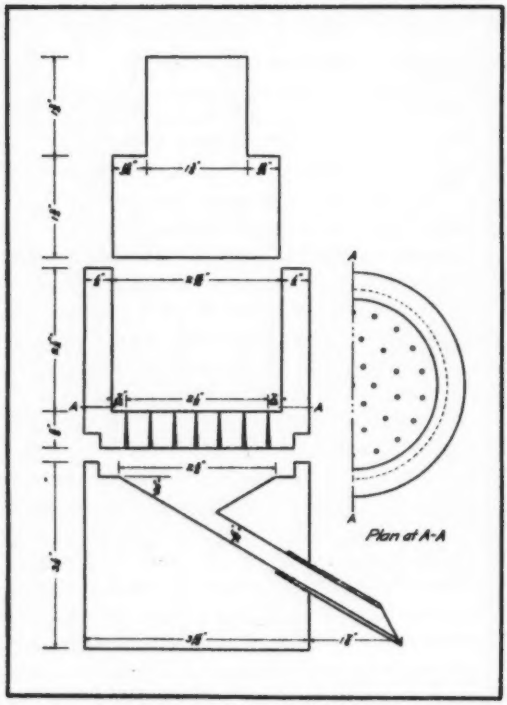


FIG. 16. *Improved type of press bowl for extraction of plant-tissue fluids.*

The proposed repetition of the experiment the next season, with younger tissues was crowded out by work on other parts of the drought investigations. It cannot yet be said with certainty, therefore, whether the method has any utility

in such studies. More succulent, younger tissues might be expected at least to yield a larger quantity of sap and thus make comparisons easier. To increase the accuracy of the extractions a new type of press bowl was designed, and although it was not used as intended for the measurement of imbibition pressures, it proved so useful and satisfactory in other parts of these investigations that it will be illustrated here.

The design of this press bowl will be clear from the drawing (Fig. 16). The diameter of the piston is adjusted to facilitate the conversion of total pressure in tons to pressure per unit area; with the diameter indicated in the figure, 1 ton = 20 atm. The piston fits the cylinder snugly, so that the leaves need not be wrapped. Only a disc of cotton of the same diameter as the cylinder is required to cover the small perforations in the bottom. The taper of these perforations prevents clogging. The fluid extract drains through the spout into any desired receptacle.

Rate of Water Loss from Cactus Segments under Various Humidity Conditions

The ability of cacti to resist drought is well known. Their water economy is associated with: (1) a highly developed root system in the surface layer of the soil, which enables them to absorb effectively the moisture from light, intermittent rains; (2) fleshy stems in which water may be stored in large quantities against periods of drought; (3) a thickened cuticle which restricts evaporation.

The imbibitional properties of the fluid expressed from the fleshy stem segments was shown by figures already given: *viz.*, with a concentration of only 5.1%, its bound-water content was 16.6%, and its viscosity was such that the ground segments had to have at least an equal quantity of water added before the juice could be pressed out with any facility, and this juice had to be still further diluted to 1.3% concentration before it could be conveniently handled. In short, a cactus segment might almost be regarded as a natural emulsoid gel. However, there are some important distinctions, notably its relatively impervious envelope, which prevent its treatment or study as a simple gel.

To determine the power of these plants to retain water against evaporation, segments of *Opuntia polyacantha* were stored in atmospheres of various relative humidities and their rate of water loss estimated. Fresh plants were obtained in July, 1925, from the dry plains of southern Alberta, through the courtesy of Mr. W. L. Jacobson, of the Department of the Interior, Irrigation Experiment Station, at Brooks. The segments were cut from the plants, their cut surfaces sealed with a mixture of beeswax and paraffin, and put into closed glass vessels of which the stoppers were sealed with vaseline (Plate II), over solutions ranging from concentrated sulphuric acid to pure water, giving 0, 25, 50, 75, and 100% relative humidity, respectively.

Four segments were stored in each vessel, but these were in some cases reduced to three or, in one case, to two, by discarding at the end of the experiment those which showed signs of disease injury. Only those which appeared to remain healthy throughout the entire course of the experiment were considered in the results. In addition, three whole plants were stored in the same well-lighted room, but under ordinary atmospheric conditions.

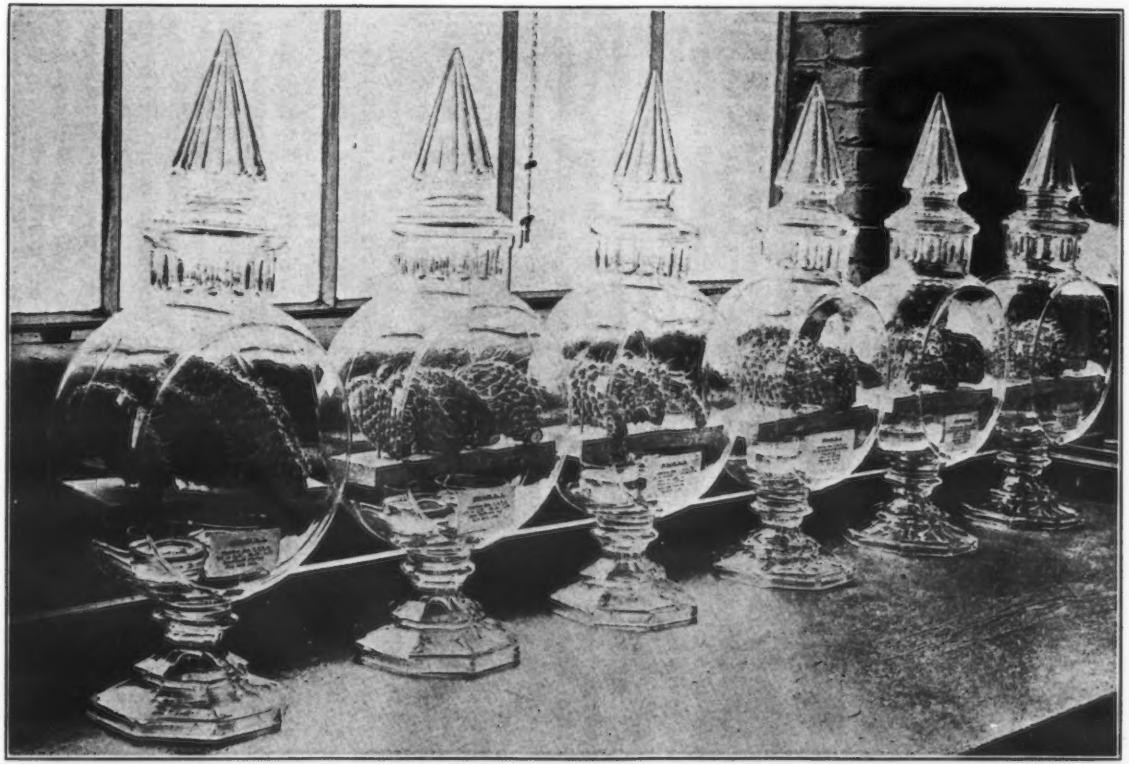
TABLE XXXI

PROGRESSIVE AVERAGE WEIGHTS OF CACTUS SEGMENTS, EXPRESSED AS PERCENTAGES OF INITIAL WEIGHTS, AND LOSS OF MOISTURE AND DRY MATTER DURING STORAGE UNDER VARIOUS HUMIDITY CONDITIONS

Conditions of storage	0% R.H.** 147 days	0% R.H. 95 days 100% R.H. 53 days	25% R.H. 95 days 75% R.H. 53 days	50% R.H. 148 days	75% R.H. 96 days 25% R.H. 52 days	100% R.H. 95 days 0% R.H. 53 days	Lab. atm. 148 days
Initial wt.* (gm.)	65.25	52.30	42.36	37.61	37.94	33.71	325.33
Time in days							
0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
1	96.6	96.4	—	—	—	—	97.6
2	93.1	93.2	—	—	—	—	95.3
3	91.5	90.9	90.4	91.2	94.4	96.1	93.2
4	89.2	88.7	—	—	—	—	91.4
5	87.7	87.4	—	—	—	—	90.1
6	86.2	86.0	84.5	85.4	90.7	93.5	88.8
8	84.4	84.5	82.7	83.7	89.6	93.0	87.1
10	82.5	82.9	—	—	—	—	85.6
13	80.7	81.8	79.5	80.8	87.9	91.8	84.0
24	76.5	78.7	76.2	77.8	86.0	90.6	81.0
35	73.4	76.8	74.4	76.7	85.4	90.5	77.3
61	70.2	74.4	71.9	74.1	82.1	90.1	72.0
73	69.3	73.5	70.8	73.1	80.7	89.6	69.2
95	67.4	69.9	68.1	—	—	88.9	—
96	—	—	—	70.5	78.4	—	67.6
97	—	70.2	—	—	—	88.3	—
98	—	70.4	—	—	—	87.8	—
99	66.7	—	—	70.2	77.7	—	—
100	—	—	68.1	—	—	—	—
101	—	70.4	—	—	—	87.2	—
102	—	70.2	67.9	—	77.4	87.1	—
104	—	70.3	67.8	—	77.2	86.9	—
108	—	70.3	—	—	76.6	86.1	—
109	65.8	—	67.7	69.5	—	—	—
115	64.8	70.2	—	—	—	84.8	—
116	—	—	67.2	68.6	75.5	—	63.9
129	62.6	69.7	66.5	67.1	74.2	82.9	61.2
139	60.0	69.1	65.5	64.5	72.4	81.5	59.0
147	58.2	—	—	—	—	—	—
148	—	67.9	64.7	59.5	70.4	80.4	57.2
Final wt. (gm.)	38.02	35.72	27.46	22.41	26.69	27.07	185.5
Final moisture content (%)	78.84	82.69	81.93	79.05	83.22	83.36	70.00
Loss of moisture (%)	45.3	34.9	36.9	43.8	30.2	20.2	52.5
Loss of dry matter (%)	22.9	26.7	27.0	22.7	26.3	16.2	−6.9

Initial moisture content taken as 84% in all cases.
**Relative humidity.*

Weighings of all segments and plants were made at intervals throughout a period of 148 days. The results for the healthy specimens are given in Table XXXI, expressed as percentages of the initial weights to facilitate



Cactus segments stored in glass vessels at various humidities.

PLATE II

comparison, since of course the actual initial weights varied. However, the actual initial and final weights are also given, being in each case the average weight per segment, or in the last column, per plant.

The initial moisture content of 20 representative segments was determined at the beginning of the experiment and found to be 84.0%. This value, for obvious reasons, had to be taken arbitrarily as the initial moisture content of all the segments used in the experiment. From the initial and final weights, and the initial and final moisture contents, have been calculated the loss of both moisture and dry matter during storage. These data are shown at the bottom of the table.

From the column headings in the table, it will be seen that in four cases the segments were transferred at the end of about 95 days from one vessel to another in which the humidity conditions were different. The purpose of this was to ascertain if the segments, like emulsoid gels, would come to new equilibria corresponding to the changed humidity conditions.

The losses of dry matter were quite high, ranging from 0.9 to 2.4 gm. per segment, or 16.2 to 27.0% of the initial content. The moisture losses ranged from 5.7 gm. to 24.8 gm. per segment, or 20.2 to 45.3% of the initial content. The actual weights lost under different humidity conditions have, of course, less significance than the percentages, since the weights of the segments varied widely. A careful scrutiny of the original figures for individual segments within a given container indicates that the relative size of segment was not an important factor in the percentage loss of either moisture or dry matter.

The figures in Table XXXI show, as expected, that the moisture losses are more affected by conditions of storage than are the dry-matter losses. The change of conditions during the course of the experiment complicates the interpretation of the results, and if the experiment is repeated this feature will be omitted. However, an examination of the tabulated figures in conjunction with the hydration curves (Fig. 18) confirms the foregoing conclusion.

The whole plants stored under the ordinary atmospheric conditions of the laboratory (which usually run from 30 to 50% relative humidity) lost more moisture than any of the segments in the closed vessels, and showed an apparent gain of 6.9% in dry matter. Even if we allow for the possibility of error in the assumption of 84% as the initial moisture content, and assume instead a loss of 25% dry matter to make the plants comparable in this respect with the segments, the percentage loss of moisture is still greater than in any of the segments stored in closed vessels. This may have been due to greater physiological activity in the plants, as they continued active growth (Plate III-A), and perhaps to the loss of a certain amount of moisture through the exposed root ends. Also, it is not impossible that a gain in dry matter may have resulted from photosynthesis. In any event, the experimental data leave no doubt of the fact that the plants finished with a substantially lower moisture content than segments stored under severer conditions.

The changes in weight of the segments during storage may be followed most readily in Fig. 17. Here, time is plotted according to the exact hour of weighing the segments, though in Table XXXI it was given only to the nearest

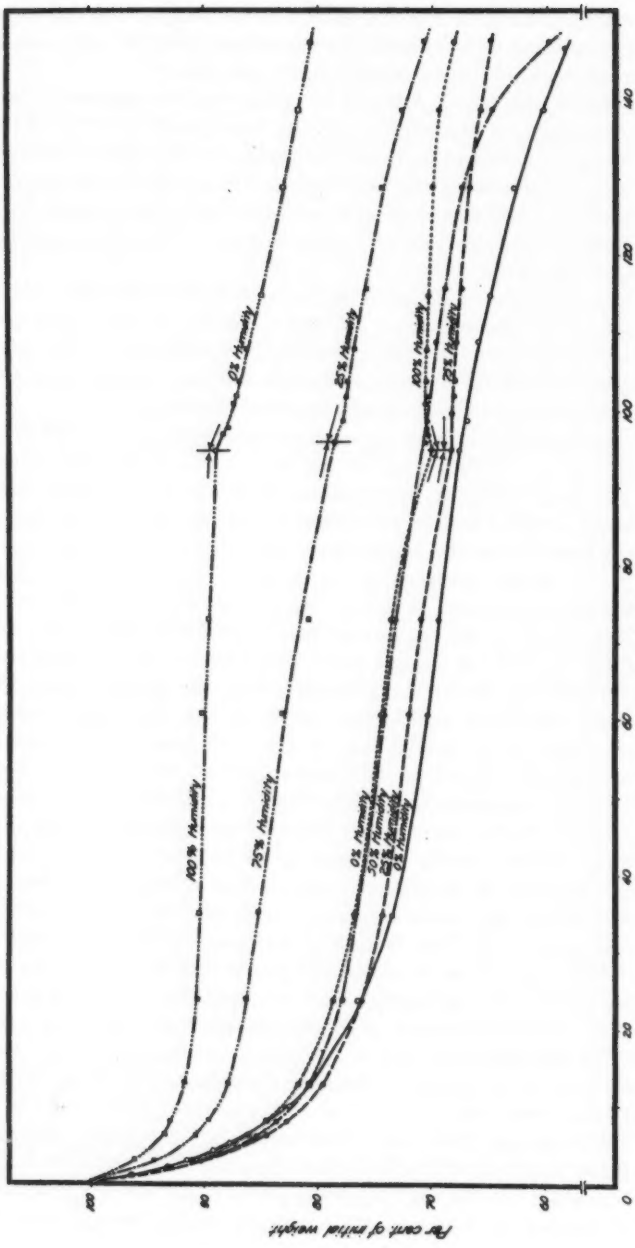


FIG. 17. *Progressive weights of cactus segments, expressed as percentages of initial weights, during storage under various humidity conditions.*

full day. The curves show that the losses were, in general, inversely proportional to relative humidity. The change in four cases in conditions at the 96th day altered the curves appreciably, but scarcely to the extent which might have been expected. The segments did not behave as non-living gels. An examination of the two cases in which humidity conditions were kept constant throughout the entire experiment (at 0 and 50% humidity) suggests that equilibrium, so far as attainable, had been reached in 95 days, and that thereafter the physiological organization of the segments began to break down, leading to increased losses probably of both moisture and dry matter. This would naturally affect the validity of all the curves beyond that point. The curve for 50% humidity falls away rather abruptly towards the end, a fact which probably explains the relatively small difference between the total loss of moisture in the two cases kept at 0 and 50% humidity throughout the entire experiment.

Since the data in Table XXXI do not make it possible to calculate the relative rates of loss of water and dry matter, the hydration curves of the segments cannot be derived exactly. However, though the percentages of dry-matter loss are quite high, the actual weights are small in comparison with the water loss. The former average 1.7 gm. and the latter 13.6 gm. per segment. Therefore no great error can result from assuming that the loss of dry matter bore a linear relation to time, and on this basis calculating the course of hydration of the dry matter during the first 95 days, when humidity conditions were constant for each group of segments. This procedure has been followed in constructing the curves in Fig. 18.

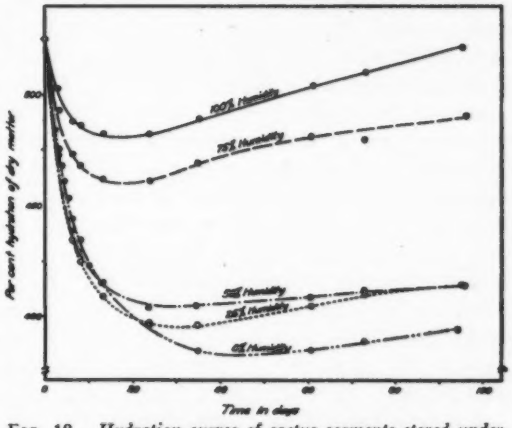


FIG. 18. *Hydration curves of cactus segments stored under various humidity conditions.*

The curves show no essential difference in initial rate of decrease in hydration at 0, 25 and 50% humidities, and surprisingly little difference in later equilibria under these conditions. At 75 and 100% humidity, however, a much higher level of hydration is maintained, and the relationship is as expected. The upward movement in all cases at the end of about a month must apparently be explained by change in temperature, a factor which was not controlled. The experiment was set up on July 24, and with the approach of autumn there came naturally a decline in temperature. At any given relative humidity, an increased hydration with lower temperature would be in accord with the behavior of emulsoid gels. Unlike a gel, however, it is probable that the extra

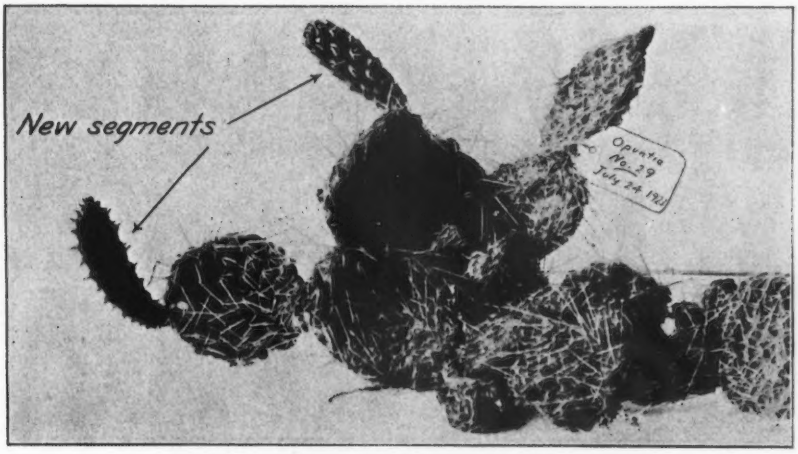
moisture was supplied from within as a product of respiration. For example, the hydration on the 95th day, of the dry matter in the segments stored at 0% humidity (which were the largest segments and therefore underwent the greatest changes in weight) represented 0.9 gm. of water per segment in excess of that contained by the same quantity of dry matter on the 61st day, when the curve reached its lowest point. But in the meantime these segments averaged a loss of 0.6 gm. dry matter each. In no case was there an increase in absolute moisture content: this declined steadily throughout the experiment. But the ratio of moisture to dry matter increased towards the end as described.

The initial loss of moisture at 100% humidity indicates that the cactus plants, although they had been collected several days earlier and shipped some 300 miles, were at the time of setting up the experiment still affected by the condition of moisture equilibrium gained when the roots were in contact with soil moisture. An ordinary emulsoid gel will shift to a lower moisture equilibrium when taken out of contact with water and placed in a saturated atmosphere. This observation and others made in the course of these investigations suggest the utility of experiments in which such properties as transpiration rate, residual moisture content of tissues, and bound water and osmotic pressure of tissue fluids, are measured with plants grown under conditions controlled in regard to moisture and temperature of both soil and air.

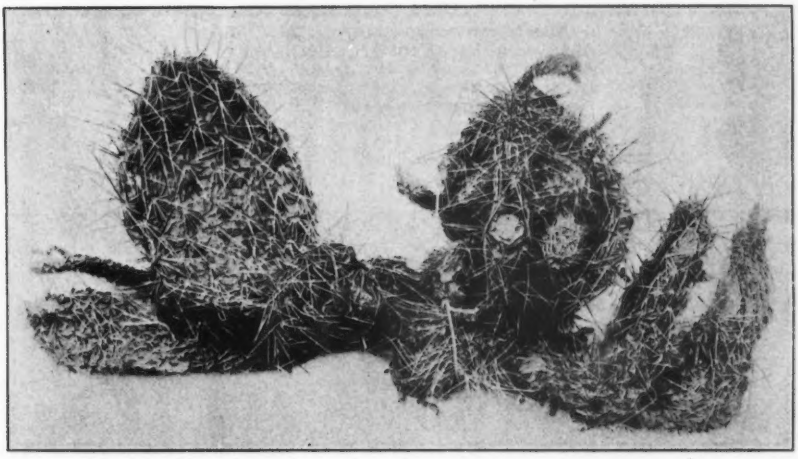
One of the striking results of this experiment is the astonishingly high moisture content of the segments after storage at 0% humidity for 147 days. This was 78.8%, as compared with an initial content of 84.0%. While the colloids were mainly responsible for the storage of this moisture, the experiment does not make it possible to say what part they played in restricting its evaporation. It is probably safe to assume that the waxy covering was more largely responsible for the latter function. It would be interesting to conduct further evaporation experiments with excised discs of cactus tissue, such as MacDougal (28a) used in swelling and shrinkage studies, or with segments from which the epidermis had been removed or made permeable by treatment without destroying the internal organization.

The vitality and longevity of both cactus plants and segments, in the absence of any external water supply, is also very remarkable. In Plate III-A is shown one of the three whole plants used in the experiment. By the end of the experiment it had developed two vigorous new segments. In Plate III-B is shown one of the same plants, photographed nearly four years after storage, which had been retained as a matter of interest to see how long it would survive. It had remained on the shelf of a store-cupboard with a glass front, facing a window in a well-lighted room, at 30 to 50% relative humidity. Each year it sent out new segments, though successive crops became gradually smaller and less vigorous. Finally it was judged to be probably dead, and when placed in water it failed to revive, but this did not happen until it had been stored three years and nine months.

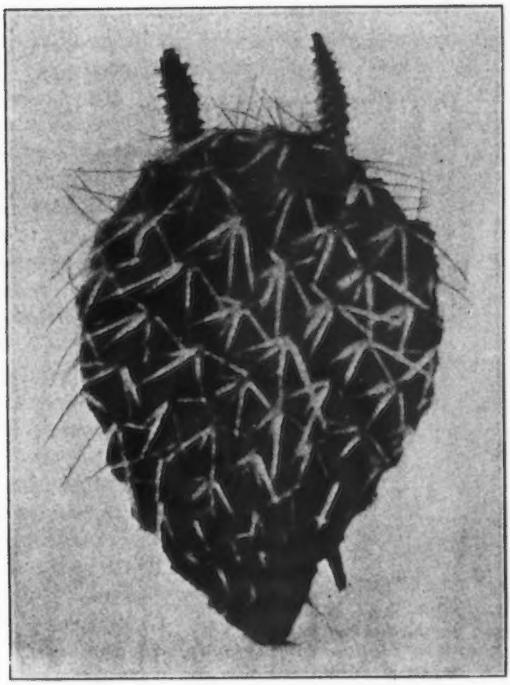
Two of the segments stored at 0% humidity for 95 days and later transferred to 100% humidity began growth under the latter conditions. One of these is shown in Plate IV-A. The new shoots appeared about a month after the transference took place.



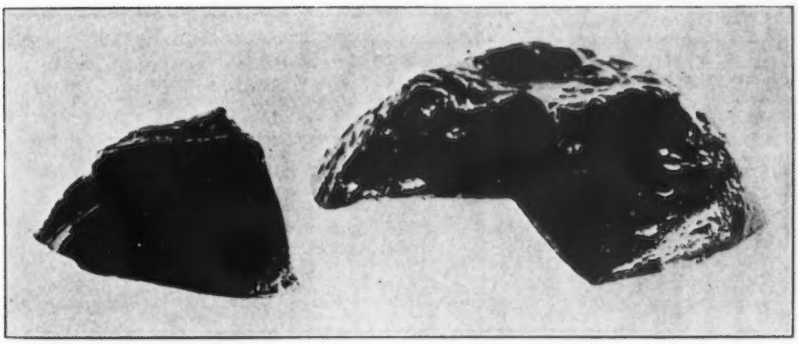
A. *Cactus plant showing new growth during storage in laboratory. (Photo Dec. 19, 1925, 148th day after storage.)*



B. *Cactus plant after nearly four years storage, during which new shoots were sent out annually. (Photo June 22, 1929.)*



A. *Cactus segment showing new growth on transference to 100% relative humidity after storage 95 days at 0% relative humidity. (Photo Dec. 19, 1925, 148th day of experiment.)*



B. *Colloidal gel formed by press juice of Agropyron tenerum.*

Rate of Water Loss from Leaves of Timothy and Western Rye Grass

On account of the striking differences between timothy, *Phleum pratense*, and western rye grass, *Agropyron tenerum*, two widely grown grasses, in drought resistance and in juice properties, it was deemed of interest to measure the relative rates of water loss from their leaves under known conditions. Triplicate samples of fresh leaves, collected from adjoining field plots of the two species on July 30, 1925, and wrapped loosely in cheesecloth, were stored at room temperature in desiccators over concentrated sulphuric acid. The leaves were weighed at intervals during a period of about 98 hr., by which time they had reached approximate equilibrium. They were then dried 48 hr. in a vacuum oven at 97-98° C. The results with the triplicate samples agreed closely, and in Table XXXII the combined weights are given, together with the relative progressive weights and the hydration of the dry matter at each time of

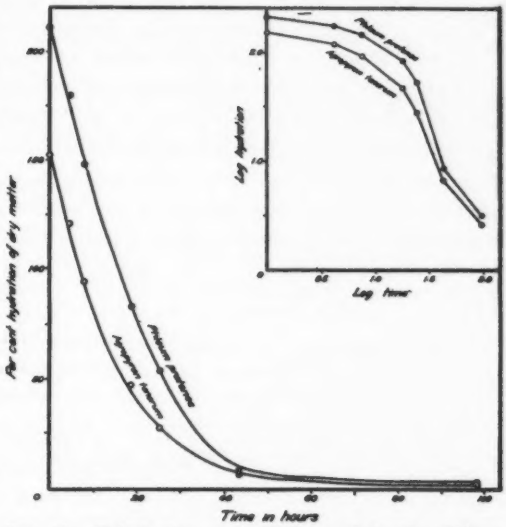


FIG. 19. *Dehydration curves of leaves of timothy, Phleum pratense, and western rye grass, Agropyron tenerum at 0% relative humidity.*

TABLE XXXII

RATE OF WATER LOSS FROM LEAVES OF TIMOTHY AND WESTERN RYE GRASS STORED AT 0% RELATIVE HUMIDITY

Time in hr.	Timothy			Western rye grass		
	Total weights 3 samples in gm.	Relative progressive weights in %	Hydration of dry matter in %	Total weights 3 samples in gm.	Relative progressive weights in %	Hydration of dry matter in %
0.0	65.29	100.0	211.3	65.69	100.0	152.8
4.28	58.74	90.0	180.1	57.42	87.4	121.0
7.80	52.09	79.8	148.4	50.60	77.0	94.7
18.80	38.48	58.9	83.5	38.37	58.4	47.6
25.25	32.33	49.5	54.2	33.24	50.6	27.9
43.53	22.83	35.0	8.9	27.75	42.2	6.8
98.53	21.65	33.1	3.2	26.67	40.6	2.6
Oven-dry matter	20.97	32.1	0.0	25.99	39.6	0.0

weighing. The progressive dehydration is also represented graphically in Fig. 19, plotted from both actual units and logarithmic values.

The results do not indicate any essential difference in the behavior of these two grasses. Neither do the logarithmic values suggest an adsorption curve such as might be expected if the action were controlled by colloids. The leaves of timothy started with a higher moisture content, but both species dried to about the same level and at about the same rate. This happened notwithstanding certain structural differences (noted below) which should have favored retention of water by the western rye grass, and the contrast in the juice properties of the tissue used, shown in Table XXXIII.

TABLE XXXIII

PROPERTIES OF TWO LEAF-TISSUE JUICES

	Conc'n %	Osmotic pressure atm.	Bound water %
Western rye	17.3	20.1	11.9
Timothy	11.7	17.5	4.7

The conditions of course were unnatural; the leaves, unlike the cactus segments, died. It is clear that structural modifications and colloidal content, in the comparative degree in which they exist in these grasses, do not function effectively in reducing rate of water loss when the living organization is broken down. If Fig. 18 and 19 are compared, it will be seen that the rates of dehydration in the grass leaves and cactus segments under similar conditions are in magnitude about as days to hours. In no circumstances, however, would we expect the grass leaves to retain moisture as effectively as the highly specialized cactus tissues.

The results of this experiment point again to the advisability of conducting experiments such as have been suggested above, with living plants under controlled conditions. They also emphasize the probable importance already indicated of the habit of maintaining a lower level of hydration observed in resistant plants, and give further point to the suggestion that the preservation of cell organization during periods of stress may be a colloidal function at least equal in importance with reduction in rate of water loss.

NOTE ON STRUCTURAL FEATURES OF WHEAT AND GRASS LEAVES

Although histological studies are not within the scope of these investigations, the importance of structural features is recognized, and microscopic observations were made of a few leaf sections of all the wheat and grass species worked with. Leaves of the distinctly drought-resistant species were found to possess well-marked structural modifications, such as thickened cuticle, sunken stomata, and specialized tissue which acts as a motor organ in the folding or rolling of leaves during periods of drought. This tissue is composed of large thin-walled cells situated at the bottom of grooves or furrows containing the

stomata, and under drought conditions it loses water rapidly, resulting in a loss of turgor and a consequent folding or rolling of the leaves. When folding or rolling occurs, the grooves in which the stomata are situated are partly or completely closed, thus reducing the rate of diffusion of water vapor into the external atmosphere. This type of adaptation was found to be well-marked in the leaves of *Bouteloua gracilis*, *Stipa comata*, and *Agropyron smithii*, all of which are very resistant to drought.

Notwithstanding the marked xerophytic features of *Bouteloua gracilis*, the moisture content of its leaf tissue collected on July 27, 1926, was found to be only 29% of the green weight. The structural modification of the leaves was not effective in controlling the absolute water loss, but was more probably effective in reducing rate of water loss during the initial period of drought, thus allowing the cells time to develop physiological resistance. Structural or mechanical features may thus be regarded as safety devices protecting against rapid desiccation while the cells are adjusting themselves physiologically for drought endurance.

A logical extension of these observations would be to compare the sizes of cells and intercellular spaces in drought-hardy and non-hardy grasses and cereals; also the nature of the cuticle, number of stomata, and character of conducting tissue. In no common farm crops, however, should we expect to find such highly developed and effective features as exist in plants like the cactus.

SUMMARY

The method of measuring the imbibition pressure of leaves by their resistance to extraction of sap under direct pressure proved unsuitable with leaves in an advanced stage of development. Whole leaves yielded juice of less than half the concentration obtained from ground leaves. An improved type of press bowl for such work is described.

The rate of water loss from cactus segments was not widely different at 0, 25 and 50% relative humidity, but greater than at 75 or 100% humidity. With an initial moisture content of 84.0%, the minimum content after 148 days was 78.8%. Cactus plants continued to grow for years in the absence of external water supply.

The rate of water loss from detached leaves of *Phleum pratense* and *Agropyron tenerum* at 0% relative humidity was essentially alike, in spite of wide differences in drought resistance and juice properties of these two grasses.

Certain structural features of drought-resistant grasses are noted.

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CYTOLOGICAL STUDIES IN THE GENUS *AGROPYRON*[1]

By F. H. Peto[2]

Abstract

Eighteen species of the genus *Agropyron* from Western Canada, Russia, Siberia, and Denmark formed a polyploid series with a basic number of seven chromosomes. Di-, tri-, tetra- and octaploid forms were found among Western Canadian species; di-, tetra-, penta-, hexa- and decaploid forms among the Russian and Siberian species; di-, tetra- and hexaploid forms among the Danish species.

The forms collected in Western Canada showed an extremely wide range of morphological variability both within and between the various species. This is probably due to natural hybridization such as has been reported between certain native species in Russia and Denmark. The cytology of these European species and their supposed hybrids has been studied here and has shown that natural crossing has probably taken place between diploid and hexaploid species in some cases and between tetraploid and hexaploid species in others. Some evidence of a similar situation among Western Canadian species has been found. Chromosome doubling, triploidy and aneuploidy occurred in certain of the latter. The relation of these phenomena to hybridization is discussed.

Introduction

The taxonomy of the genus *Agropyron* is in a very unsatisfactory condition. Authorities differ widely in their concepts of the species within this genus. This undoubtedly is due to the wide range of variability existing within the so-called species and also to the large number of intergrading types that occur between these species. In some instances the extent of the variability has never been reported and in others some of the intermediate types have been classified as distinct species. These taxonomic difficulties extend to the seeds; analysts and seed merchants are unable to separate the desirable and undesirable forms with reasonable accuracy. Comparative morphology cannot be relied upon entirely to solve these taxonomic difficulties; additional evidence, provided by ecological, genetical and cytological studies, appears to be necessary. The results reported in this paper indicate the contribution to this end which may be made by cytological methods.

There is relatively little information available on the cytology of wild or cultivated grasses. Evans (7) in 1926 reported counts in various species of *Lolium* and *Festuca*. Davies (5) a year later announced counts for *Dactylis glomerata*. Church (3, 4) published in 1929 the results of cytological studies in 31 species of the tribes *Festuceae*, *Aveneae*, *Agrostideae*, *Chlorideae*, *Phalarideae*, *Paniceae*, and *Andropogoneae*.

A review of the literature revealed only one instance where any cytological work had been done on the genus *Agropyron*. Stolze (24) included in the *Triticum* group the counts of *Triticum* (*Agropyron*) *repens* L. and reported 21 chromosomes in pollen mother cells and 42 in somatic cells.

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Contribution from the Department of Field Crops, University of Alberta. Based upon a thesis presented to the University of Alberta in partial fulfilment of the requirements for the degree of Master of Science.

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The early cytological findings in this investigation were reported by the writer (20) in a short article in which the chromosome counts of six species of *Agropyron* were given. These species were found to fall into three groups with haploid chromosome numbers of 7, 14 and 21. Since then, the number of species studied has been enlarged to 18, with haploid numbers forming a complete polyploid series extending from 7 to 35.

Morphological Variability

The morphological variability in *A. tenerum* was recognized by Malte (17) in observations made at Edmonton, Canada, where he found forms of western rye grass occurring in great profusion. Kirk (14) collected seed of 150 individual plants of this species within a radius of 10 miles of Saskatoon and remarks that one is impressed with the variability exhibited in this material. He states, "Some of the more obvious characters in which the strains differ are color, width and texture of leaf; fineness, height and strength of stem; length and density of spike; earliness and productivity".

Raunkiaer (21) made a detailed morphological study of the native species of *Agropyron* from different localities in Denmark. He differentiated between the various forms by means of small morphological characters which he called "isoreagents". He studied 12,000 plants of *A. repens* from different localities, and observed the pubescence, color, etc. He found that there seemed to be no definite relation existing between these different characters and the environment. He studied similarly *A. junceum* L. Beauv., *A. litorale* (Host) Dum., and interspecific crosses between the three species.

A study of the native species of *Agropyron* growing in Alberta indicated a wider range of variability than hitherto reported in the literature. This was especially true of *A. tenerum* and *A. richardsonii*. There appeared in nature such a complete gradation of forms between these two species that it was nearly impossible to determine which species certain of these intermediate forms more nearly resembled. Plate I-A illustrates this condition. These spikes were collected in Alberta in 1929 from wild plants. Spikes No. 1 to 5 are fairly typical *A. richardsonii*, varying only in length and color of empty glumes, while No. 25 to 28 are typical *A. tenerum*. Spikes No. 6 to 24 exhibit a complete range of variability between the typical members of the two species. This variation is particularly noticeable in length, density, color, and unilateral condition of the spikes, and in length of awns.

A similar degree of variability can be observed between the species *A. smithii* and *A. dasystachyum*. This is illustrated by the spikelets in Plate II. No. 1 to 3 are *A. smithii* forms which vary in color, size, length of awn and pubescence. No. 4 to 6 are *A. smithii molle* which differ from typical *A. smithii* in being faintly pubescent. No. 7 to 12 illustrate various spikelet forms of *A. dasystachyum*. No. 9 is nearest the typical form. No. 11 and 12 show two forms which closely resemble *A. griffithsii*, although they were identified at the Grass Herbarium, United States Department of Agriculture, as *A. dasystachyum*.

A. repens is a species introduced from Europe which has become well established, especially in the older settled districts, where it has become a

primary noxious weed. The variability found in the spikes of this species is illustrated in Plate I-B. The first four spikes represent forms found growing under similar conditions near Emerson, Manitoba. The remainder were collected in various parts of Alberta and represent a wide variation in length and density of spike, number of florets per spikelet, pubescence of the rachis, and color of the lemma and palea.

An attempt to explain the wide range of variability outlined above will be made later in this paper, taking into consideration the parallel conditions reported in European countries, as well as the cytological findings in this investigation.

Materials and Methods

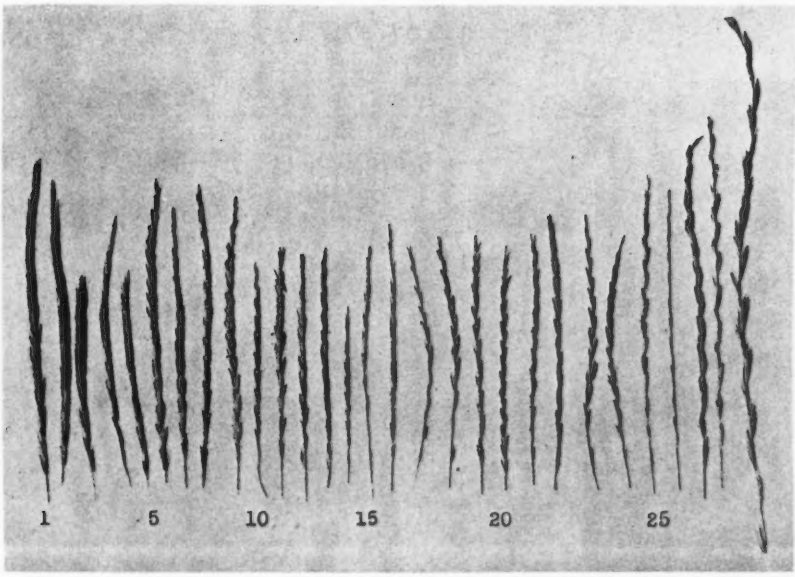
The plant material used in this investigation was obtained from various sources. The early studies were made on a number of plants collected by W. Robinson, a graduate student in the Department of Field Crops of the University of Alberta. During the summer of 1929 a large collection of native plants and seeds was obtained by the author in a field survey of the genus *Agropyron* throughout Alberta. Additional seed samples of both native and introduced species were procured from the Universities of Saskatchewan, Manitoba and California and the Dominion Range Experimental Station at Manyberries, Alberta. A number of introductions were made from the following institutions and countries: the Botanical Gardens of Tiflis, Caucase, Georgie, U.S.S.R.; the Experimental Station, Omsk, Siberia; the Experimental Station, Krasnyi Kut (Gov. Saratov) U.S.S.R.; the Botanical Gardens of Copenhagen, Denmark.

The plants were grown in the greenhouse in small pots for root-tip study, and in larger wooden boxes for pollen mother cell study. They were kept growing vigorously throughout the winter by the use of artificial lighting for part of the night. It was observed that the plants were more thrifty during the winter if given a rest period. This was done by putting them outside and allowing them to harden off during October and November.

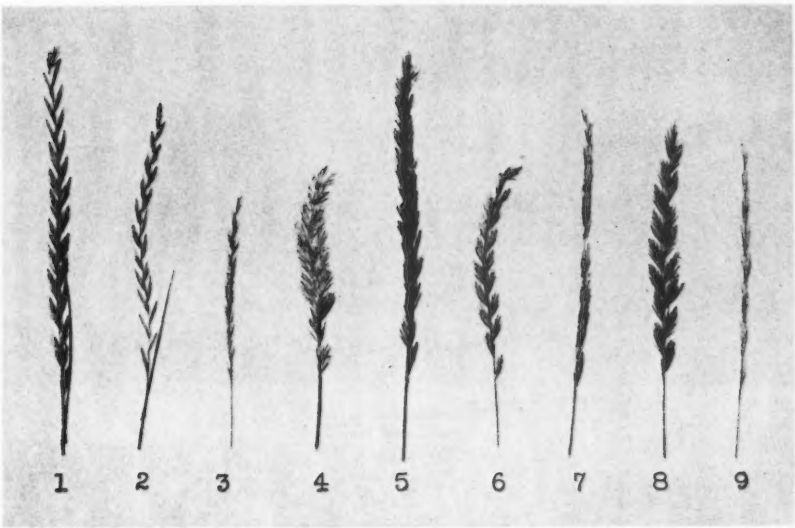
The proper stage for pollen mother cell preparations was quickly ascertained by preparing temporary mounts by Belling's aceto-carmine method (2). One or two anthers were placed on a slide and covered with a few drops of strong aceto-carmine solution which contained a trace of ferric hydrate. A cover glass was placed over them and gently tapped sufficiently to burst the anthers and allow the pollen mother cells to exude. These were stained sufficiently in a minute or two, so that the chromosomes could be distinguished. When the proper stage was observed, the florets from the same position in adjacent spikelets were used for making permanent mounts.

For the preparation of permanent mounts, the anthers with the enclosing lemma and palea were fixed in Carnoy's 6-3-1 solution for two minutes and then for 24 hr. in Zenker's fluid (15). The sections were cut about 20 μ thick. Newton's iodine-gentian-violet method proved very valuable for pollen mother cell preparations, especially in conjunction with Zenker's killing and fixing agent. It seemed particularly well suited for studying the various prophase

PLATE I



A
1-5. *Typical A. richardsonii.*
6-24. *Intermediate forms.*
25-28. *Typical A. tenerum.*



B.
Spike forms of A. repens.

1-3. *A. smithii.*
4-6. *A. smithii molle.*
7-12. *A. dasystachyum.*

stages in addition to giving excellent chromosome counts in the heterotypic metaphase. It also had the advantage of being quicker than Heidenhain's iron-haematoxylin method.

Karpechenko's killing and fixing solution used in conjunction with Heidenhain's iron-haematoxylin was found to be most satisfactory for root-tip preparations. The iodine-gentian-violet stain did not give good results with preparations of this kind.

The accompanying chromosome figures were prepared from camera lucida drawings. A Zeiss 1.4 mm. apochromatic objective and 20× ocular were used in all cases. This gave a magnification of 2800 on the original drawings which have been reduced slightly for publication.

Cytology of the Species

A. tenerum Vasey.—Thirty-seven plants were used in the cytological study of this species; they represented a very wide range of variable and intermediate forms as illustrated in Plate I-A. With one exception, the root-tips of these plants had 28 chromosomes (Fig. 1-1). The shape of these chromosomes was in general characteristic of all the species. They were very long with single or double bends. The extent of flexure in individual chromosomes did not appear to be constant and there were no marked differences in size and shape of any of the chromosomes that would be of value in identifying the species.

The meiotic behavior was observed in seven of these plants, and it appeared to be normal with the exception that in a few cases slight lagging of one or two chromosome pairs was observed in the heterotypic anaphase. This lagging did not appear to be sufficient to prevent these chromosomes being included in the daughter nuclei. The drawing in Fig. 1-2 shows 14 bivalent chromosomes at the heterotypic metaphase.

One plant was abnormal both in chromosome number and behavior throughout meiosis. In general appearance it was very similar to typical *A. tenerum*, except that it bore awns about 5 mm. long and seemed to be more vigorous. This plant was found to have the triploid complement of 21 chromosomes in its root-tips, and mitosis appeared to be perfectly normal (Fig. 1-3). The behavior during meiosis was extremely irregular. The chromosomes evidently failed to pair normally in the prophase, since from 13 to 17 univalents and from two to four bivalents with occasional trivalents were observed at the heterotypic metaphase. These bivalents and trivalents appeared to exhibit very weak telosynapsis, quite distinct from the bivalents in normal plants which exhibit parasynapsis. In Fig. 1-4 there appear to be 16 univalents, one bivalent and one trivalent chromosome at the heterotypic metaphase. In the heterotypic division the univalents wandered presumably at random to the poles while the bivalents aligned themselves at the equatorial plate. The condition is shown in Fig. 1-5. In this figure there are three bivalent chromosomes at the equatorial plate, while eight univalents are clustered at one pole and seven at the other. The distribution, however, was not always so uniform as in this cell, and the resulting daughter cells were often of unequal size and

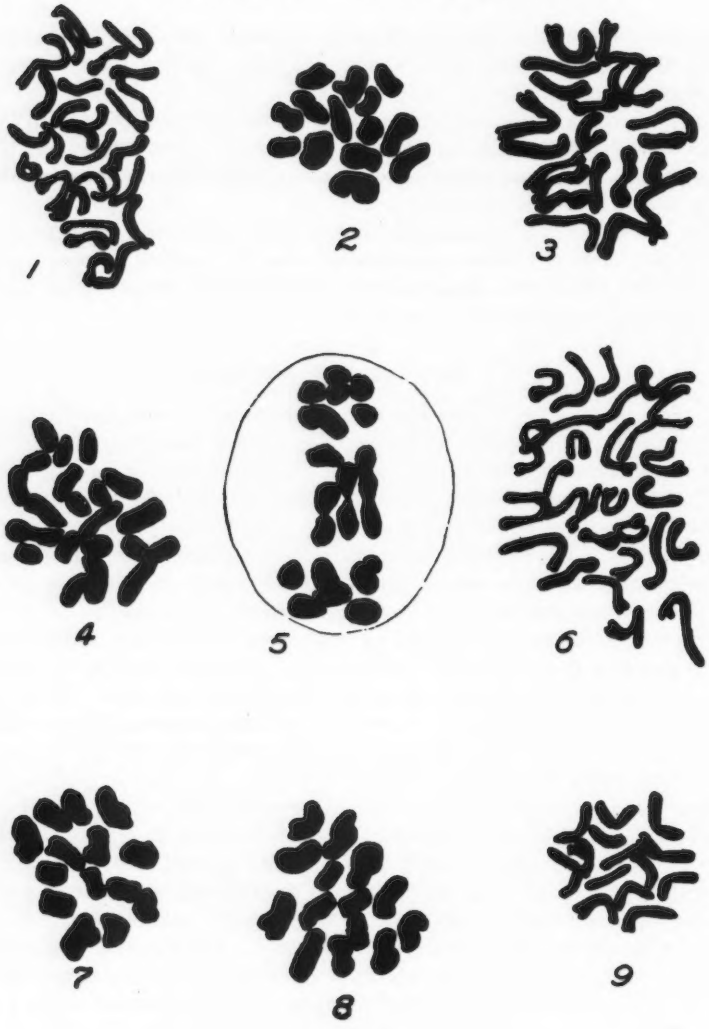


FIG. 1.

1. *A. tenerum, 28 somatic chromosomes.*
2. *A. tenerum, heterotypic metaphase, 14 bivalents.*
3. *A. tenerum, triploid form, 21 somatic chromosomes.*
4. *A tenerum, triploid form, heterotypic metaphase.*
5. *A. tenerum, triploid form, heterotypic anaphase, 3 bivalents and 15 univalents.*
6. *A. richardsonii, 28 somatic chromosomes.*
7. *A. richardsonii, heterotypic metaphase, 14 bivalents.*
8. *F_1 hybrid A. richardsonii x A. tenerum, heterotypic metaphase, 14 bivalents.*
9. *F_1 hybrid A. richardsonii x A. tenerum, homotypic metaphase, 14 univalents.*

contained varying numbers of chromosomes. In the homotypic division the chromosomes apparently divided equationally. The resulting tetrads were very abnormal and were probably sterile since this plant produced no seed.

A. richardsonii Schrad.—All the variable forms of this species referred to earlier in this paper, were examined cytologically, and the degree of external variability was certainly not reflected either in meiotic behavior or chromosome number, since the root-tips of 35 of these plants all contained 28 chromosomes (Fig. 1-6). A pollen mother cell study of a number of these plants revealed regular meiotic behavior, and clear counts of 14 were obtained in both the heterotypic and homotypic divisions (Fig. 1-7).

A. caninum (L.) R. & S.—There has been some controversy as to whether this European species is distinct from *A. richardsonii* of America. In order to determine their relationship, introductions were made of *A. caninum* from the Botanical Gardens of Copenhagen, Denmark. The only decided morphological distinction between these two species is that *A. caninum* does not have the unilateral condition of the spike so common in *A. richardsonii*. Root-tip preparations of *A. caninum* gave many clear counts of 28 chromosomes and the meiotic behavior appeared to be regular. It therefore appears to be impossible to distinguish these species by cytological methods.

Hybrids between *A. richardsonii* and *A. tenerum*.—Crosses were made between these species during the summer of 1926 by W. Robinson (22). The F_1 plants were sterile, a condition which indicated that the cross had been effected. These plants resembled the *A. tenerum* parent and were more vigorous than either parent. The examination of the pollen mother cells did not show any of the irregularities, such as incomplete synapsis or lagging chromosomes, which are typical of most hybrids. The appearance of the bivalent chromosomes in the heterotypic metaphase is illustrated in Fig. 1-8. Fig. 1-9 shows the chromosomes in the homotypic metaphase. These chromosomes more closely resemble those in somatic tissue. The bivalent chromosomes of the heterotypic metaphase differ in being much thicker; they are rectangular in general outline and show the indentations preliminary to the disjunction of the synaptic mates.

A. dasystachyum (Hook.) Scribn.—The two most important species in the short grass plains of Southern Alberta are *A. dasystachyum* and *A. smithii*. These species are usually closely associated in the field. Their foliage and root systems are very similar in appearance but the typical spikes have quite distinct characteristics, as shown in Plate II.

Fifteen plants from different collections of *A. dasystachyum* were examined cytologically; all had 28 somatic chromosomes (Fig. 2-1). A study was made of the pollen mother cells in one plant and no irregularities were observed throughout meiosis. The various prophase stages were exceptionally clear in these preparations. Three of these stages are illustrated in Fig. 2-2, 2-3 and 2-4. Fig. 2-2 shows the diplonema stage in which the synaptic mates in some cases appear to be twisted around each other, while in others they are only weakly conjugated at terminal synaptic points. Diakinesis is illustrated in Fig. 2-3. Here the synaptic mates have become more intimately associated,

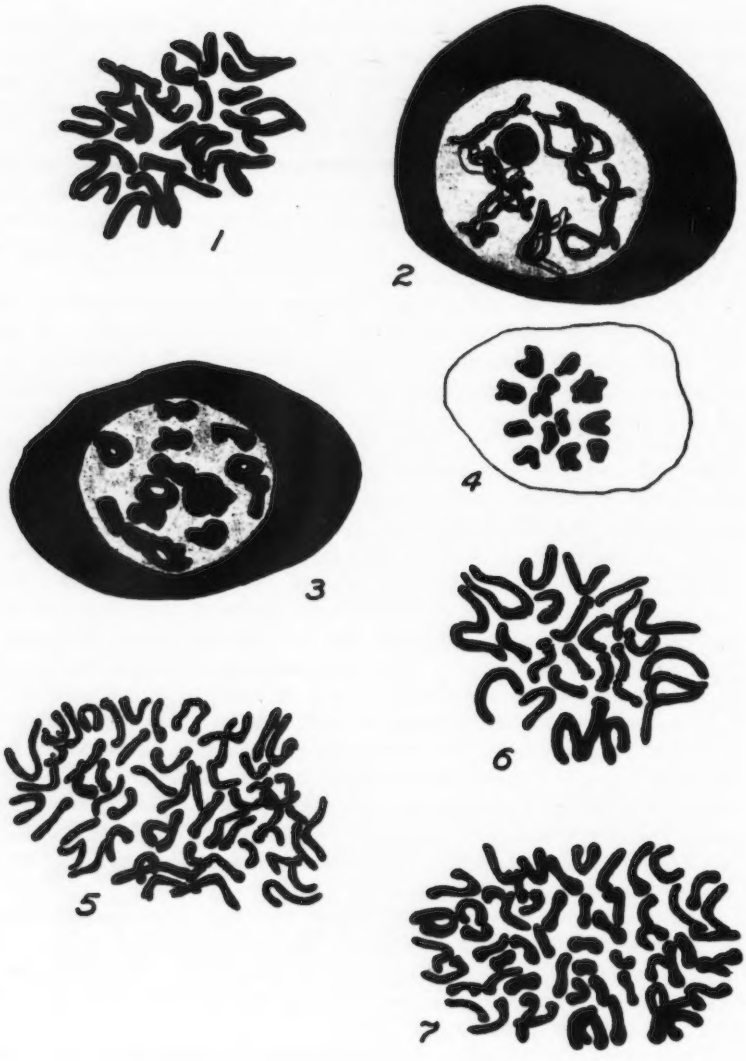


FIG. 2.

1. *A. dasystachyum, 28 somatic chromosomes.*
2. *A. dasystachyum, diplonema stage in heterotypic prophase.*
3. *A. dasystachyum, diakinesis stage in heterotypic prophase.*
4. *A. dasystachyum, heterotypic metaphase, 14 bivalents.*
5. *A. smithii, 56 somatic chromosomes.*
6. *A. smithii molle, 28 somatic chromosomes.*
7. *A. smithii molle, 56 somatic chromosomes.*

although many do not exhibit complete synapsis throughout their entire length, and hence present a ringed appearance. The chromosomes are much shorter and thicker than those shown in Fig. 2-2 and 14 bivalents can readily be counted. In Fig. 2-4 the chromosomes have reached the metaphase and are much more compact. Indentations at the ends of the chromosomes are the only visible indications of their bivalent characteristic. The nuclear membrane and the nucleolus have completely disappeared at this stage. From these preparations it would appear that conjugation between homologues is parasynaptic.

A. smithii Rydb.—It might be expected from the apparent close relationship of *A. dasystachyum* and *A. smithii* that they would have the same chromosome number, especially since there appears to be a gradation of forms between the two species. This was not the case, however, since all of the 10 strains of *A. smithii* examined had 56 somatic chromosomes (Fig. 2-5).

A. smithii molle (S. & S.) Jones.—This subspecies can only be distinguished morphologically from typical *A. smithii* by the very fine pubescence which covers the glumes and lemmas and in this respect it is intermediate between *A. smithii* and *A. dasystachyum*. Two of the four plants studied cytologically had 28 somatic chromosomes while the other two had 56 chromosomes. These counts are shown in Fig. 2-6 and 2-7. The root-tip cells of the plant containing 56 chromosomes are slightly larger, but contain slightly smaller chromosomes of the same general shape as the 28-chromosome form. There do not appear to be any external morphological characteristics by which these plants with the different chromosome numbers can be distinguished. This similarity in spike type between forms having 28 and 56 chromosomes is shown in spikelets 5 and 6 of Plate II.

A. repens (L.) Beauv.—This species was studied extensively. All the native plants investigated gave clear counts of 21 bivalents in pollen mother cells (Fig. 3-1). Slight lagging of one pair of chromosomes at the heterotypic anaphase was observed in a few cells of all the plants studied. Root-tip counts were made of nine variable forms of *A. repens* collected in Western Canada. All had 42 chromosomes.

Five plants of *A. repens* (L.) Beauv. introduced from Caucase, Georgie, U.S.S.R., were studied. Three of these plants gave counts of 42 somatic chromosomes (Fig. 3-2). Numerous clear counts of 35 were obtained from one of the plants (Fig. 3-3), while another contained either 34 or 35 chromosomes. The possibility of this being a segregating population following a cross between *A. repens* and some species with a lower chromosome complement will be discussed later.

A strain of *A. repens* obtained from the Botanical Gardens of Copenhagen, Denmark, was found to have 42 somatic chromosomes.

A. repens (L.) var. *glaucescens* Engl. was introduced from Caucase, Georgie. Root-tip counts on four of these plants showed 42 chromosomes.

A. griffithsii Scribn. & Smith.—The plants studied of this species were collected in the southwestern part of Alberta. They exhibited considerable variability in awn length and degree of pubescence of the lemmas and glumes.

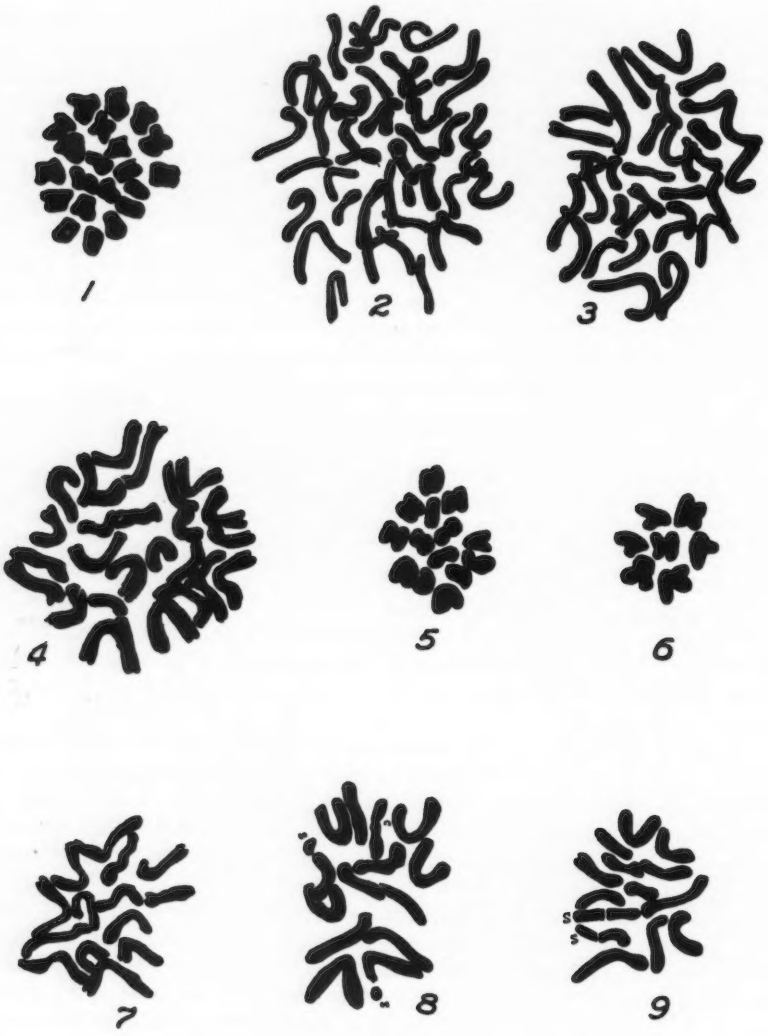


FIG. 3.

1. *A. repens, heterotypic metaphase, 21 bivalents.*
2. *A. repens, 42 somatic chromosomes.*
3. *A. repens, hybrid form, 35 somatic chromosomes.*
4. *A. griffithsii, 28 somatic chromosomes.*
5. *A. griffithsii, heterotypic metaphase, 14 bivalents.*
6. *A. spicatum, heterotypic metaphase, 7 bivalents.*
7. *A. spicatum, 14 somatic chromosomes.*
8. *A. spicatum, 14 somatic chromosomes, two satellites and one constriction.*
9. *A. spicatum, 16 somatic chromosomes. Note the chromosomes (S) which have probably become segmented.*

Only a few plants were examined cytologically, but these all gave root-tip counts of 28 chromosomes (Fig. 3-4). This number was checked in pollen mother cell preparations of one plant and clear counts of 14 bivalent chromosomes were observed in the heterotypic metaphase (Fig. 3-5). The writer in a previous publication (20) reported a plant of this species as having seven bivalent chromosomes at the heterotypic metaphase. This plant was later identified at the Grass Herbarium, United States Department of Agriculture, as *A. spicatum*.

A. spicatum (Pursh.) Scribn. & Smith.—This species is indigenous to the southwestern part of Alberta. It is the only native species that has been found to have the haploid chromosome number of seven (Fig. 3-6). A study of the root-tips proved to be very interesting, since satellites, chromosome constrictions and chromosome segmentations were observed. Five plants were examined cytologically; two of these showed none of the above irregularities and had 14 somatic chromosomes in all the counts (Fig. 3-7). In the other three plants, the majority of the cells had 14 chromosomes, but a high percentage appeared to have one to three extra chromosomes. Many of the 14-chromosome cells showed deep constrictions and satellites. Cells with varying chromosome numbers and degrees of segmentation appeared together in the same section. In Fig. 3-8 is shown a cell with two satellites (S) and one deep constriction (C). The extra chromosomes seemed to have arisen through a segmentation of certain of the chromosomes at the constriction points. Fig. 3-9 shows two chromosomes in which this may have taken place, the segments not having been shifted out of alignment. This hypothesis is supported by the observation that the cells which have the higher chromosome number seem to have an increased number of shorter chromosomes.

Satellites were observed in many of the cells but this condition was also variable in the same plant. Furthermore, some of the satellites did not appear to have any chromatic connection with the chromosomes, and it is likely that they were small segments of the chromosomes which had become separated at terminal constrictions.

Additional data will have to be procured before it can be definitely concluded that this process of segmentation is actually occurring in the living cells. It is possible that the constriction may be affected by the killing and fixing solutions, so that they become more pronounced and even separated, but this would not explain their repeated occurrence in only certain plants of this species.

A. dagnae Grossh.—Only one plant grew from the seed of this species which was obtained from Caucase, Georgie, U.S.S.R. The root-tip cells of this plant were examined and gave clear counts of 14 chromosomes (Fig. 4-1).

A. cristatum J. Gaertn.—This species is indigenous to central and northern Europe and Asia. A number of introductions have been made from Russia and Siberia. These include an extremely variable number of forms, many of which are difficult to distinguish from species introduced as *A. sibiricum* and *A. desertorum*.

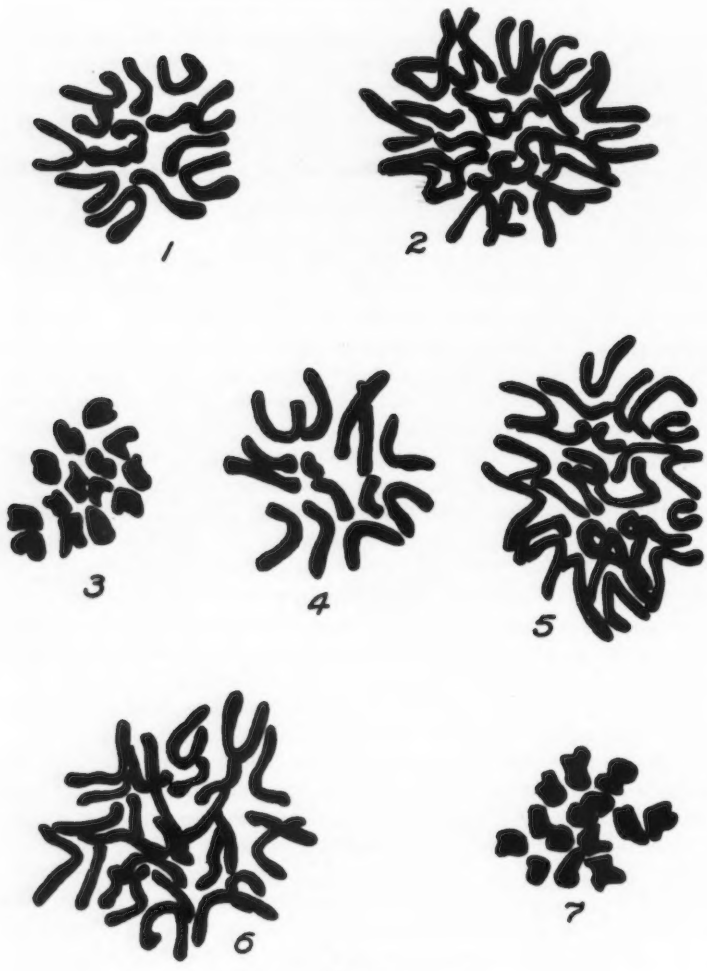


FIG. 4.

1. *A. dagnae, 14 somatic chromosomes.*
2. *A. cristatum, 28 somatic chromosomes.*
3. *A. cristatum, heterotypic metaphase, 14 bivalents.*
4. *A. cristatum, 14 somatic chromosomes.*
5. *A. sibiricum, 28 somatic chromosomes.*
6. *A. desertorum, 28 somatic chromosomes.*
7. *A. desertorum, heterotypic metaphase, 14 bivalents.*

The cytology of *A. cristatum* is especially interesting as strains have been found with different chromosome numbers. Introductions from Caucase, Georgie, the University of California, the Montana Agricultural Experiment Station, and strains growing at the University of Alberta all gave root-tip

counts of 28 (Fig. 4-2). Pollen mother cell preparations on some of this material showed 14 bivalents in the heterotypic metaphase (Fig. 4-3); introductions from the Experimental Station at Omsk, Siberia, had 14 somatic chromosomes in all the plants studied (Fig. 4-4); while introductions from the Experimental Station at Krasnyi Kut included three strains showing 14, and one showing 28 somatic chromosomes. Additional data are needed before it can be determined whether or not a given strain has always a constant number. There appeared to be no morphological characteristics which would clearly distinguish between the 14- and 28-chromosome forms, but certain plants with the latter number seemed to resemble *A. sibiricum* and *A. desertorum*. If additional taxonomic and cytological evidence corroborates this observation, then only those forms that belong to the 14-chromosome group may reasonably be considered typical *A. cristatum*.

One plant consistently gave clear counts of 29. It was from a strain obtained from Dr. S. E. Clarke of the Dominion Range Experimental Station at Manyberries. This plant differed from the average in being less vigorous and bearing very small awnless spikes. This case of aneuploidy will provide interesting material for further study.

A. sibiricum (W.) Eichw.—Introductions of this species were obtained from Caucase, Georgie, and Omsk, Siberia. These plants all had 28 somatic chromosomes (Fig. 4-5).

A. desertorum.—The Botanical Garden of Copenhagen has this species listed as a variety of *A. sibiricum*, while the specimens received from the Krasnyi Kut Experimental Station were listed as distinct species. Seven strains from this station and one from Copenhagen gave clear root-tip counts of 28 chromosomes (Fig. 4-6). Pollen mother cell counts of 14 bivalent chromosomes were made on a plant obtained from the Manitoba Agricultural College (Fig. 4-7).

A. elongatum.—Dr. L. E. Kirk, of the University of Saskatchewan, obtained this species from Russia, and supplied seed to the writer. Cytological examination of two plants revealed clear counts of 70 chromosomes in the root-tips (Fig. 5-1). This is only the second species in the *Gramineae* to be reported with a chromosome number of this magnitude. Church (4) reported a decaploid count of 35 bivalent chromosomes in *Andropogon scoparius*.

A. villosum Link.—This species introduced from Denmark is striking morphologically because of its resemblances to common wheat, particularly with reference to the non-adherence of lemma and palea to the ripe caryopsis. Pollen mother cell preparations were made of two plants. A large number of counts were obtained at diakinesis (Fig. 5-2). Seven bivalent chromosomes are shown, in addition to the nucleolus which is still heavily stained at this stage.

A. junceum (L.) Beauv.—This is another introduction from the Botanical Gardens of Copenhagen. Raunkiaer (21) observed this species to have less variability than *A. repens*. The plants that were examined of this strain gave 28 somatic chromosomes (Fig. 5-3). The hybridization of *A. junceum* with certain other Danish species will be referred to in the discussion.

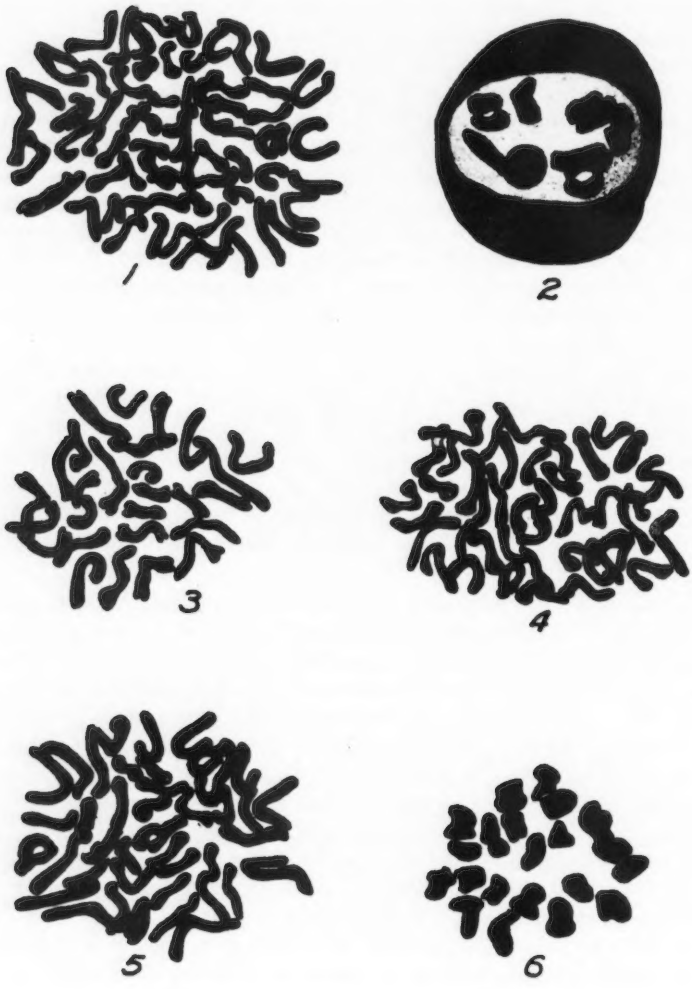


FIG. 5.

1. *A. elongatum, 70 somatic chromosomes.*
2. *A. villosum, 7 bivalents at diakinesis.*
3. *A. junceum, 28 somatic chromosomes.*
4. *A. glaucum, 42 somatic chromosomes.*
5. *A. obtusiusculum, 42 somatic chromosomes.*
6. *A. pungens, heterotypic metaphase, 21 bivalents.*

A. glaucum R. & S.—This is a Danish species obtained from the Botanical Gardens at Copenhagen. Root-tip examination of three plants of this species gave clear counts of 42 chromosomes (Fig. 5-4).

A. obtusiusculum Lange.—One strain of this species was introduced from the University of California and two from the Botanical Gardens at Copenhagen. These three strains gave root-tip counts of 42 chromosomes (Fig. 5-5).

A. pungens (Pers.) R. & S.—This species was introduced from Sutton & Sons, Reading, England. Pollen mother cell preparations were made from three plants and 21 bivalent chromosomes were observed in the heterotypic metaphase (Fig. 5-6). Slight lagging of two or three pairs of chromosomes was frequently observed in the heterotypic anaphase.

Discussion

The chromosome numbers have been determined in 18 species of *Agropyron*. With the exception of two cases of aneuploidy, they form a polyploid series with di-, tri-, tetra-, penta-, hexa-, octa- and decaploid numbers. The counts obtained for the various species are summarized below, and are listed under the countries from which the species were obtained.

	n	*2n*
Species collected in Western Canada:		
A. spicatum Pursh.	7	14
A. tenerum Vasey (hybrid plant) (21 univalents)	—	21
A. tenerum Vasey	14	28
A. richardsonii Schrad.	14	28
A. griffithsii Scribn. & Smith	14	28
A. dasystachyum (Hook.) Scribn.	14	28
A. smithii molle (S. & S.) Jones	—	28
A. smithii molle (S. & S.) Jones	—	56
A. smithii Rydb.	—	56
Species introduced from Russia and Siberia:		
A. dagnae Grossh.	—	14
A. cristatum J. Gaertn.	—	14
A. cristatum J. Gaertn.	14	28
A. sibiricum (W.) Eichw.	—	28
A. desertorum	14	28
A. repens (hybrid plant)	—	34 & 35
A. repens (L.) Beauv.	21	42
A. repens (L.) var. *glaucescens*	—	42
A. elongatum	—	70
Species introduced from Denmark:		
A. villosum Link.	7	—
A. caninum (L.) R. & S.	—	28
A. junceum (L.) Beauv.	—	28
A. sibiricum var. *desertorum*	—	28
A. glaucum R. & S.	—	42
A. repens (L.) Beauv.	—	42
A. obtusiusculum Lange	—	42
Species introduced from England:		
A. pungens (Pers.) R. & S.	21	—

In general, the Canadian, Russian and Danish species of *Agropyron* are quite distinct morphologically. It is therefore interesting to note that the species from these countries fall into very similar polyploid series with the same basic chromosome number of seven. This possibly indicates that they have evolved along parallel lines.

The polyploid series reported in this investigation is very similar to that found in most of the genera of *Gramineae* which have been studied. Sax (23) and others found the wheats to fall into three definite polyploid groups—diploid, tetraploid and hexaploid. Tschermak and Bleier (25) produced an octoploid form from crosses between *Aegilops ovata* with *Triticum dicoccoides* and *T. durum*. Griffee (8) reported the groups in the genus *Hordeum* with 7, 14 and 21 haploid chromosomes. Kihara (12) reported the same series in *Avena*. Church (3) studied the meiotic phenomena in a number of species of the tribes *Festuceae*, *Aveneae*, *Agrostideae*, *Chlorideae* and *Phalarideae*, and obtained with few exceptions counts which showed the same polyploid series as reported for the cereals. Evans (7) found haploid chromosome numbers of 7 and 21 for species of *Festuca* and of seven for *Lolium perenne*. The tribes *Paniceae* and *Andropogoneae* were studied by Church (4). The species fell into the same series of multiples with the exception of *Andropogon scoparius*, which gave a decaploid count of 35 haploid chromosomes, the highest number hitherto reported for any species of *Gramineae*. The basic number varied somewhat, however, in these tribes, since five species were reported with haploid chromosome counts of nine and 15 species with haploid counts of 10 or multiples of 10.

There are numerous instances cited in the literature where hybridization is believed to be responsible for the initiation of new polyploid and aneuploid forms. This is believed to be the case in the wheats. Aase (1) presents a summary of most of the recent evidence on the cytology of wheat and aegilops hybrids and their phylogenetic relationships. An hypothesis was developed regarding the origin of the various wheats by a study of chromosome homology, as indicated by the extent of chromosome conjugation between the various chromosome sets. In this manner Aase (1) shows that *Triticum vulgare* contains three distinct sets of seven chromosomes. Two of these sets are found in emmer and a third is found in *Aegilops cylindrica*. She states that this does not necessarily indicate that the vulgare wheats have resulted from the hybridization of *Ae. cylindrica* and emmer, but that it is more probable that *Ae. cylindrica* and *T. vulgare* have one parent in common.

Huskins (10) in his studies of fatuoid oats makes this statement: "Probably hybridization and polyploidy are related phenomena in the origin of wheat and oats, and are equally and jointly responsible for the origin of speltoids and fatuoids as well as many of the peculiar features discovered in hybridization studies".

Church (3) in his studies on 16 species of the tribes of *Gramineae* mentioned above concludes that, "Polyploidy or cytological abnormalities of the maturation divisions or both are considered as evidence of the hybrid origin of all the species except *Phalaris arundinaceae*".

Jeffrey (11) believes that hybridism has clearly played a large role in the acceleration of the processes of evolution. He states, "Where interspecific crossing is possible, there is often clear evidence of its presence in the form of a high degree of variability, accompanying a considerable manifestation of sterility in the gametic cells, particularly the pollen".

Cytological evidence has been used in a number of instances in recent years to aid in the solution of some of the more perplexing taxonomic difficulties. This is particularly true in the genus *Rosa*. A recent paper by Erlanson (6) of the University of Michigan is worthy of review, since the situation she found has many points in common with that found in *Agropyron*. Both of these native genera are widely distributed and extremely variable, and both contain certain wild species between which there is no clear line of morphological demarcation. Erlanson studied 107 wild roses from stations in North America. The majority of the specimens on cytological examination fell into three classes with diploid, tetraploid and hexaploid numbers, the basic number being seven. One specimen was octoploid, two were triploid, and three aneuploid with the somatic number not a multiple of seven. Many of the diploid roses exhibited irregularities during meiosis. Incomplete pairing at diakinesis, lagging chromosomes, and polyspory were frequently observed in some individuals; these were believed to be spontaneous hybrids produced by the crossing of different species.

The cytological findings presented by the foregoing workers emphasize the importance of hybridization in initiating morphological and cytological variability. The cytological evidence of natural hybridization in the Western Canadian species of *Agropyron* is not abundant. This by no means indicates that hybridization may not be responsible for a great deal of the variability. According to Malte (17), Robinson (22) and Kirk (14) the species of *Agropyron* are probably highly self-fertilized. This decreases the chances for natural hybridization and increases the homozygosity of those types that have arisen in this way. Natural inbreeding in the progeny of hybrid plants would have the tendency to eliminate rapidly disadvantageous recessives and abnormalities in meiosis, which might be perpetuated much longer in cross-fertilized populations. It might, therefore, be expected that the cytological evidence of hybridization would not be so plentiful in *Agropyron* as in cross-fertilized genera such as *Rosa*.

The role played by hybridization in species formation in *Agropyron* cannot be determined until further studies have been carried out on the meiotic behavior of a large number of natural forms as well as of artificial hybrids. However, certain phenomena have been observed, that apparently can only be satisfactorily explained by assuming interspecific hybridization.

The abnormal triploid plant of *A. tenerum* which has 21 somatic chromosomes may possibly be a hybrid between *A. tenerum* which has 14 bivalent chromosomes and some species of the seven-chromosome group. Synaptic pairing is for some reason deficient and as a result only from two to four bivalent chromosomes and an occasional trivalent are observed in the heterotypic metaphase. The unpaired univalents apparently wander to the poles at

random. This is typical hybrid behavior similar to that found in hybrids arising from crosses between *Zea mays* (10 pairs) and *Euchlaena perennis* (20 pairs) in which Longley (16) reports the appearance of univalents, bivalents and trivalents at meiosis. The 30 chromosomes in the F_1 hybrid are irregularly distributed in the two maturation divisions.

Cytological methods may be inadequate to determine the extent of natural hybridization between such species as *A. tenerum* and *A. richardsonii*. Morphological studies demonstrate a complete range of intermediate forms between them, yet it seems impossible to find any evidences of hybridity by cytological examination. These naturally occurring intermediate forms all appear to be fertile and have the same number of somatic chromosomes. Pollen mother cell studies have been made on only a few plants, but artificial hybrids between these two species appear to have perfectly normal meiotic behavior. Evidently these two species have a very close phylogenetic relationship and therefore do not exhibit the meiotic irregularities so common to interspecific hybrids.

It is difficult to account for the occurrence of both tetraploid and octoploid plants of *A. smithii molle*. This species is commonly found in the same region as *A. smithii* and *A. dasystachyum*, and in many characteristics is intermediate between the two. One might assume that the octoploid form had originated by hybridization of these two species but such an assumption does not necessarily follow, since *A. smithii* already has 56 chromosomes.

The fact that different chromosome numbers have been observed in plants of *A. smithii molle* does not necessarily mean that these plants belong to different species. Navashin (19) in referring to chromosome doubling in *Crepis*, states that forms arising in this way should not be designated as new species, since the main differences between the speciesdepend not so much upon differences of chromosome number as upon qualitative differences of chromosome constitution. There does not, however, seem to be unanimity of opinion upon this point. Heilborn (9) in connection with a study on the chromosome numbers in *Draba* makes this statement: "If two similar forms are seen to have different chromosome numbers, it is a proof that these forms are different, and ought to be separated".

A similar situation was found in the introduced species *A. cristatum*, in which a number of strains had 14, while others had 28 somatic chromosomes. Here again certain of these strains with different chromosome numbers closely resembled each other morphologically, but in general it was observed that the 28-chromosome strain more closely resembled *A. sibiricum* and *A. desertorum* than those of the 14-chromosome strain. An attempt to explain this apparent doubling of chromosome number will be made in the following paragraphs.

Numerous indications of interspecific hybridization in the genus *Agropyron* have been observed in European countries. Troitzky (26) states that in the steppe Haredja (Central Transcaucasia) in places where *A. cristatum* J. Gaertn. grows in association with *A. repens* (L.) P.B. var. *glaucescens* Engl., specimens were observed transitional in all their recognizable characters between the two above-mentioned species, and forming moreover a series of very gradual

transition from the one to the other. Of these specimens, those occupying an intermediate position between *A. repens* and *A. cristatum* cannot be distinguished morphologically from *A. sibiricum* Eichw. Hitherto the latter species has not been met with in Transcaucasia. It is however scattered over southeastern Russia, western Siberia and Turkestan. Troitzky concludes that this phenomenon may be attributed to a secondary formation of *A. sibiricum* through hybridization or it is a convergent formation identical to the existing species.

The various species referred to by Troitzky (26) were introduced and examined cytologically and the results fully corroborate his conclusions. *A. repens* var. *glaucescens* has 42, and certain forms of *A. cristatum* have 14 somatic chromosomes. These two species would produce gametes containing 21 and seven chromosomes. On crossing, these would result in progeny with 28 somatic chromosomes, which is exactly the count obtained for *A. sibiricum*. If this hypothesis can be proved to be true it will explain also the apparent occurrence of two different chromosome numbers in strains of *A. cristatum*, *i.e.*, the 28-chromosome forms of *A. cristatum* are really hybrid forms that have probably arisen as the result of natural hybridization between 14-chromosome strains of *A. cristatum* and *A. repens*.

The difficulty in this hypothesis is that it is not usual for 14- and 42-chromosome forms to cross and, in subsequent generations, yield progeny that has consistently 28 chromosomes. Melburn and Thompson (18) crossed *T. monococcum* with *T. spelta* and observed that this 7- and 21-gamete combination contained from zero to five bivalents with the mode around three to four. When *T. aegilopoides* was used as the seven-chromosome parent, Kihara and Nishiyama (13, cited by Aase, 1) observed from six to 10 conjugates, including one or two trivalents. Seven bivalents were rather common. Since *A. repens* var. *glaucescens* and *A. cristatum* appear to cross readily in nature, one would expect to find a much stronger homology between their chromosomes than would be exhibited in the difficult artificial wheat cross reported above. Homologous chromosomes of different sets of *A. repens* would apparently have to conjugate in order to insure fertility and constant chromosome number in the resulting progeny. This theoretical difficulty can only be solved by crossing these two species and studying the conjugation between their chromosome sets.

Additional evidence on the occurrence of natural hybridization among certain Russian species of *Agropyron* is found in one strain of *A. repens* in which two plants were found, one with 34 and the other with 35 somatic chromosomes. These plants are possibly the progeny of natural hybridization between *A. repens*, with 21 haploid chromosomes, and some species such as *A. sibiricum*, with 14 haploid chromosomes.

Raunkiaer (21) reports that in Denmark many types were found which he believed to be hybrids between *A. junceum* and *A. repens* and which were very similar to *A. obtusiusculum* Långe. He further reports forms of which he believes some to be hybrids between *A. junceum* and *A. litorale*, and others to be hybrids between *A. repens* and *A. litorale*.

The chromosome numbers have been established for three of these Danish species: *A. junceum* 14 (n), *A. repens* 21 (n) and *A. obtusiusculum* 21 (n). Raunkiaer believed that natural hybridization occurred between the two first-mentioned species and that the resulting hybrids were similar to *A. obtusiusculum*. A study of the meiotic behavior in the progeny of this cross between species of different chromosome numbers would be valuable. One would expect to find, however, that individuals with the chromosome number of *A. obtusiusculum* would be very infrequent. Seed of *A. litorale* was not obtained from Denmark, but Raunkiaer believed this species to be synonymous with *A. pungens*, which gave counts of 21 (n) chromosomes. If this is the case, then the reported cross between *A. junceum* and *A. litorale* was also between species with different chromosome numbers, and would provide good material for cytological study.

Conclusions

A wide range of morphological variability was observed both within and between the native species of *Agropyron*. A complete series of intermediate forms occurred between certain species closely associated in the same environment. This was found to be particularly true in two instances: *A. richardsonii* and *A. tenerum; A. smithii* and *A. dasystachyum*.

Similar situations were reported among species of *Agropyron* in Denmark by Raunkiaer (21) and in Russia by Troitzky (26). They attributed the occurrence of intermediate types to natural hybridization between distinct species, basing their conclusions solely on circumstantial evidence provided by ecological and morphological observations and by analogical deductions from artificial crosses between various species indigenous to the localities under consideration.

The cytological studies carried out in this investigation provide more direct evidence that natural hybridization does occur among species of *Agropyron*. Troitzky (26) concluded that certain forms resembling *A. sibiricum* had arisen as the result of natural hybridization between *A. repens* and *A. cristatum*. This conclusion is corroborated by the chromosome counts of these species. Hybridization between *A. repens* and *A. cristatum* with gametes of 21 and 7 chromosomes respectively would probably give rise to progeny with 28 somatic chromosomes, which is exactly the count found for *A. sibiricum*.

Forms of *A. cristatum* were introduced from Russia with 14 and 28 somatic chromosomes. This species was extremely variable, but in general the plants of the higher counts resembled *A. sibiricum* and *A. desertorum* to some extent. In the light of the reported natural hybridization between *A. repens* 21 (n) and *A. cristatum* 7 (n), it seems probable that only the forms containing 14 somatic chromosomes were typical *A. cristatum*, while the 28-chromosome forms were probably of hybrid origin.

Further evidence of natural hybridization between the Russian *Agropyron* species was found in a strain of *A. repens* which contained two plants with somatic numbers of 34 and 35 respectively, while the remainder had the normal number of 42. These plants were probably the progeny of a cross between

A. repens and some species of the 28-chromosome group. The chromosome numbers of the Danish species indicated that here again there may be considerable natural hybridization between species belonging to the tetraploid and hexaploid groups.

The morphological variability observed in the genus *Agropyron* in Western Canada would indicate that considerable natural crossing has taken place, although there has not been a great deal of cytological evidence accumulated to support this view. The intermediate types between *A. richardsonii* and *A. tenerum* were examined cytologically but they failed to reveal any of the usual hybrid symptoms. This was also true of artificial hybrids between these species. There is evidently such complete homology between the chromosome sets of such species that the usual meiotic abnormalities do not occur on hybridization. One plant resembling *A. tenerum* was found, however, to have the triploid complement of 21 chromosomes, the normal number being 28. The abnormal behavior throughout the meiosis in addition to the intermediate number suggested hybrid origin.

The significance of the variations in the somatic chromosomes of *A. spicatum* is very uncertain. Satellites, chromosome constrictions and chromosome segmentations were observed in certain plants of this species. This condition was variable in any individual plant. Segmentation appeared to have taken place at the constriction points, resulting in higher counts in some cells.

The various species of *Agropyron* could not be distinguished by chromosome morphology, since no striking differences in this regard were noted between them. The chromosome number of *A. repens* may be of value in distinguishing this noxious weed from economic forms, since this is the only wild-growing species in Western Canada that has 42 somatic chromosomes.

To sum up, it may be stated that morphological and cytological evidences of hybridity have been found in many of the native and introduced species of *Agropyron*. Not only is natural hybridization apparently occurring between species with the same chromosome number, but also frequently between species with different chromosome numbers. Several morphologically indistinguishable forms have different chromosome numbers, while many distinct species have the same chromosome number. While these findings may in a sense add to the complexity of the taxonomist's problem, they at least account for the wide range of variability found both within and between the so-called species of *Agropyron*. They also provide a sound basis for further cytological investigations and for economic breeding work with this genus.

Acknowledgment

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STUDIES ON REACTIONS RELATING TO CARBOHYDRATES AND POLYSACCHARIDES

XXXIII. THE SYNTHESIS OF POLYSACCHARIDES BY BACTERIA AND ENZYMES[1]

By F. C. Harrison[2], H. L. A. Tarr[3] and Harold Hibbert[4]

Abstract

The synthesis of a polysaccharide from simpler carbohydrates both by the agency of *Bacillus mesentericus* Trevisan and *Bacillus subtilis* Cohn has been studied. The action of both is apparently specific in that they only bring about polysaccharide formation from carbohydrates containing a terminal fructofuranose residue in their molecule. A similar synthesis can be carried out by using the corresponding enzyme preparation from *Bacillus mesentericus*.

Introduction

In connection with the sugar industry it has long been recognized that the juice of the sugar cane and sugar beet is peculiarly susceptible to fermentative changes, variously designated as "slimy", "mucilaginous" and "gummy" by different authors. Pasteur (22) first showed the slimy fermentation of carbohydrates to be bacterial in origin, and to the resulting gummy material he assigned the formula $C_{12}H_{20}O_{10}$. Smith (27) and Dodson (6) were among the first to prove conclusively the relationship between bacteria and the deterioration of crystalline sugar, though Shorey (24), at a somewhat earlier date, had observed deterioration in Hawaiian crystalline sugar which was brought about by a mould, *Penicillium glaucum*. This latter author failed to note the presence of any bacteria in the sugar which he examined.

The number of micro-organisms which can produce deterioration in cane and beet sugar, and their products, is very large; in fact, several types of fermentation may occur simultaneously. The gummy products formed can usually be grouped conveniently according to the sugars which they yield upon hydrolysis. For example, "dextrans" yield glucose; "galactans", galactose, and "levans", fructose.

One of the most important fermentations liable to occur in the cane, or beet sugar factory, is the "mucilaginous" fermentation whereby "dextran", a substance which hydrolyses completely to glucose, is formed from sucrose by the action of certain organisms, one of the most important of which is *Leuconostoc mesenteroides*. Durin (7) considered this product to be a cellulose, but Scheibler (16, p. 461-468) first established its true nature as a product closely related to starch and dextrin*.

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* *An investigation dealing with the structure of dextran is in progress, as well as of the products obtained by the action of Bacterium xylinum on carbohydrates.*

It is also possible to obtain a cellulose-like material in considerable amounts from sucrose by a type of fermentation which Brown (3) found to be aërobic. He described the organism yielding the cellulose-like material as being probably identical with *Bacterium xylinum* of Browne (4).

There are also numerous references in the literature to the production of galactans, arabans, etc. by bacteria when grown in a suitable nutrient medium containing carbohydrate. Thus Smith has described *Bacterium sacchari* (28, 31), *Rhizobium limosospongiae* (34) and *Bacterium atherstonei* (32) all of which produce "galactan" gums. Organisms which produce complex gums of the arabin group, yielding both arabinose and galactose upon hydrolysis, are not uncommon, and appear to be associated chiefly with the gum flux of fruit trees. Thus Smith has described *Bacterium acaciae* (29) and *Bacterium metarabinum* (29), both of which produce an arabin gum, yielding arabinose and galactose upon hydrolysis; and *Bacterium pararabinum* (30) which produces a pararabin gum, also yielding galactose and arabinose upon hydrolysis. Smith (33) also studied the formation of gum by *Rhizobium leguminosarum* and found that the substance produced from sucrose by this organism yields both glucose and galactose upon hydrolysis. Buchanan (5) observed that *Bacillus radicicola* (*Rhizobium radicicola*) forms a mucilaginous material from 15 different carbohydrates, as well as from glycerol and salts of certain organic acids. He found the gummy material in each instance had the same general characteristics, and assumed the gum to be of a dextran nature.

In addition to the dextrans, glucosans and other types of bacterial polysaccharides mentioned above, it is possible to obtain levulans in which the fructose component of the sucrose molecule has polymerized under the influence of certain bacteria. Laxa (16, p. 469-471) found that a polysaccharide produced by a strain of an organism which he termed *Clostridium gelatigenosum*, upon hydrolysis, yielded *d*-fructose. Maassen (16, p. 471-473) showed that the formation of certain Leuconostoc-like masses in sugar solutions was brought about by an organism which he called *Semiclostridium commune*. This species, and three others which he isolated from different sources, were characterized by their ability to yield a levulan. One type of levan was described by Lippmann (18) who investigated a mucilaginous substance formed in a vat containing sugar beet juice and identified it as a fructosan, m.p. 250° C.; $\alpha_D - 221°$. It was readily soluble in hot calcium hydroxide, and upon oxidation yielded no mucic acid. The freshly precipitated material when in moist condition was readily soluble in hot and cold water, and when precipitated from alcoholic solutions formed a white, amorphous, highly hygroscopic powder. In this condition it was soluble in hot, but insoluble in cold water. One-half per cent was sufficient to cause the solution to gel on cooling. It did not reduce Fehling's solution, was precipitated by lead acetate in concentrated solutions, and on hydrolysis with dilute acids yielded *d*-fructose quantitatively.

Smith (26) isolated, from commercial sucrose, an organism closely related to *Bacillus vulgatus*, which, because of its power to form a gummy substance (levan), he named *Bacillus levaniformans*. This was best cultivated in a nutrient medium containing 10% sucrose, suitable nutrient salts and 0.1%

peptone. Organisms of this and other closely related species, all of which are resistant spore-formers, were found to be of widespread occurrence in commercial sugars (27). Steel (37) studied the chemical properties of the levan prepared by Smith (26) from sucrose and found that it yielded only fructose upon hydrolysis. It differed from Lippmann's levulan (18) in specific rotation, melting point, and the fact that its aqueous solutions did not gelatinize on cooling. Moreover, while certain chemical and physical relationships were shown by levan, inulin and starch, the first named was found to differ from both the latter compounds in certain important respects.

The levan prepared by Smith (26) from sucrose could not be obtained from glucose, fructose, lactose or maltose (36). This fact, together with the relatively marked power to invert sucrose possessed by *Bacillus levaniformans*, led Smith and Steel (36) to assume that levan could only be formed from the so-called nascent fructose and glucose resulting from the inversion of sucrose. They thus assumed the action of invertase as necessary to the formation of levan, although this point was not proven.

Smith (25) also discussed the formation of a product similar to levan which he obtained by the action of *Bacterium eucalypti* (a non-spore-forming organism) which he isolated from the gummy exudate of the tree *Eucalyptus stuartina*. He stated that, "the activity of the organism was tested upon sucrose, but the gum was formed naturally from raffinose, the manna of the eucalypt, with which the gum was mixed".

From the tissues of a seedling of *Eucalyptus hemiphloiae* Smith (35) isolated an organism capable of forming in sucrose solutions a gum similar to levan. He termed this *Bacillus hemiphloiae* because it differed from *Bacillus levaniformans* in not forming spores, and from *Bacterium eucalypti* in producing gas in certain sugar solutions.

Kopeloff and Kopeloff (13) concluded that the "moulds" are by far the most important single group of micro-organisms normally occurring in sugars of all descriptions. Kopeloff et al. (12) succeeded in preparing levan from sugar solutions of widely different concentration, up to the saturation point, by means of enzyme preparations obtained from the mould *Aspergillus sydowi*. They believed that the levan was formed from the nascent fructose and glucose, resulting from the inversion of the sucrose, and that the fructose reacted to a far greater extent than the glucose in levan formation. Very little levan was formed when normal fructose (fructo-pyranose) and glucose (gluco-pyranose) were employed. Appreciable concentrations of acidity or alkalinity inhibited the activity of the enzyme, the optimum reaction being about pH 7.0.

Owen (20) noted that the relatively high resistance to heat shown by the spore-forming organisms of the *Subtilis-mesentericus* group accounted for the presence of such bacteria in the finished sugar; he also confirmed Smith's findings (27) in regard to the prevalence of such organisms in commercial sugars. Most of the organisms isolated by Owen were able to form levan in nutrient sucrose solutions similar to those employed by Smith (26). Owen, however, disagreed with both Kopeloff et al. (12) and Smith (26) in that he believed levan was formed directly from the sucrose molecule, and that the

inversion of sucrose into nascent fructose and glucose had no connection with the actual formation of levan. Experiments in which he employed yeast, and later (21) yeast invertase itself, in conjunction with the levan synthesizing organisms, with the idea of augmenting the formation of nascent fructose and glucose, showed that the presence of reducing sugars liberated by the invertase appeared actually to inhibit the production of levan. Naturally this tends to substantiate his theory of levan production direct from sucrose; but it must not be forgotten that the invertase produced by yeast cells may differ from that produced by the bacterial cells, especially in regard to the optimum pH required for its activity. Moreover the nascent fructose liberated from sucrose by yeast invertase would probably not be available to the bacterial cell for polymerization into levan, because in aqueous solution it presumably undergoes immediate, spontaneous transformation into the inactive (pyranose) form. It would thus seem far more probable that the sucrose molecule is inverted by the bacterial invertase within the confines of the cell, where the nascent fructose is immediately polymerized to the levan polysaccharide by some levan-forming enzyme, the levan subsequently diffusing into the surrounding medium. It would, however, be extremely difficult to obtain experimental proof of such a theory. At present, therefore, no definite statement can be made regarding the method of formation of levan by the bacterial cell.

Taggart (38) made a chemical analysis of the levan prepared by Owen (20) and obtained results very similar to those of Smith and Steel (36).

Beijerinck (1) showed that many bacilli produce a peculiar colloid reaction, an emulsion being formed which surrounds the colonies of these organisms when grown on neutral, or faintly alkaline, nutrient agar containing either sucrose or raffinose. The enzyme responsible for this reaction, which he named viscosaccharase, could be separated along with several other enzymes by filtering a culture of *Bacillus mesentericus vulgatus*, and adding alcohol to the filtrate.

An organism, provisionally designated gummobacter, has been shown by Fernbach and Schoen (8) to form from sucrose a gummy material very similar to levan. This organism, the development of which is hindered by oxygen, does not yield gummy products from carbohydrates other than sucrose, and they therefore believed the gum to be formed from "levulose naissant". More recently (9) they claim to have shown definitely that the gum is obtained only from the fructose portion of the sucrose molecule.

At this stage it would not seem premature to emphasize one outstanding fact found in reviewing the literature dealing with the levan type of gum formation; namely, the evidence submitted by Smith (25) and Beijerinck (1) that levan is produced from sucrose and raffinose and not from other sugars. The full significance of this fact is referred to later.

Discussion of Results

Experiments in these laboratories were initiated with the intention of studying the formation of levan and other types of polysaccharide by bacteria and their enzymes, and determining the constitution of these synthetic com-

pounds in the light of recent developments in our knowledge of carbohydrate chemistry. An account of the methods used in determining the chemical constitution of levan is to be found in the following communication (11).

Owen (20) showed that, in general, levan is synthesized by the *Subtilis-mesentericus* group of micro-organisms, and that these are of widespread occurrence in commercial raw and refined sugars. This work was confirmed in the present investigation by the isolation, on sucrose-agar by the plate method, of a number of cultures from commercial brown sugar, followed by inoculation of sucrose broth from the colonies thus formed. The relative power of the organisms to form levan was determined by a comparison of the precipitate formed by inoculating a similar volume of sucrose broth, incubating the solutions at 37.5° C. for six days, and then pouring the reaction product into five times the volume of ethyl alcohol, made just alkaline to phenolphthalein. The most active culture thus indicated was purified by the plate method, and classified as *Bacillus mesentericus* Trevisan, following the systematic classification of the Society of American Bacteriologists (2).

Preparation of Levan

Nutrient sucrose-broth solutions (20%) were inoculated with this culture, and by subsequent successive precipitations from 95% ethyl alcohol, followed by electrodialysis, a quantity of levan sufficient for the chemical examination was obtained, the yield being approximately 51 gm. of levan per litre of the original culture solution. The chemical investigation of this product showed it to be a polymer of 2:6 anhydrofructo-furanose (11).

Experiments to determine which carbohydrates were capable of yielding levan were then carried out. Earlier work, especially that of Kopeloff et al. (12) and of Smith (26), tended to show that so-called nascent fructose, as liberated in the inversion of sucrose, is necessary for levan formation. It seemed highly probable that the nascent fructose of these authors was, as it is now known, a fructo-furanose, and that this only, and not the pyranose form could be polymerized to give the 2:6 fructose-anhydride levan. This view was confirmed experimentally.

Nutrient solutions of a variety of sugars were prepared so that each ultimately contained 10% of the sugar, 0.1% peptone, 0.2% Na_2HPO_4 and 0.5% KCl in distilled water. These solutions were sterilized by filtration instead of by heat, as it was feared that the latter method might cause some decomposition of the carbohydrate. Each solution was then inoculated with a loopful of a fresh sucrose-broth culture of *Bacillus mesentericus*, incubated 10 days at 37.5° C., and the levan precipitated at the end of this time with 95% ethyl alcohol. The results showed definitely that levan formation only occurred with sucrose and raffinose, and not with melezitose, lactose, maltose, xylose, glucose or fructose. Similar results were obtained when a pure culture of *Bacillus subtilis* Cohn was employed in the place of *Bacillus mesentericus*, the former organism having been isolated from a laboratory stock culture in which it had appeared as a contamination and classified according to systematic classification of the Society of American Bacteriologists (2).

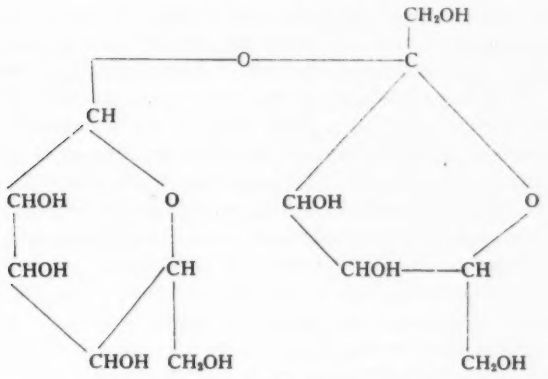
As previously stated, the work on the chemical structure of levan has shown it to be a polymer of a 2:6 anhydrofructo-furanose (11). This fact seemed of particular significance inasmuch as both sucrose and raffinose are known to contain the fructo-furanose residue in their molecules (10). However, since melezitose also contains this same grouping (40) the negative result obtained appears somewhat puzzling. A comparative study of the structural formulae of melezitose (17, 40), sucrose and raffinose (10), however, at once suggests a possible explanation of the apparent discrepancy (see page 455).

Both sucrose and raffinose have a terminal fructo-furanose group, while in melezitose there is a central anhydro-fructose molecule attached at each end to a glucose molecule. This fact would seem to explain the failure of the levan-forming organisms to synthesize levan from melezitose. This discovery is of special interest in view of the fact that yeast invertase will not attack melezitose (15). Also, the assumption has been made that yeast invertase hydrolyzes sucrose by acting on the fructose half of the molecule; it is known that it also hydrolyzes gentianose, stachyose and raffinose, all of which are closely related to sucrose in that they contain a terminal fructo-furanose grouping.

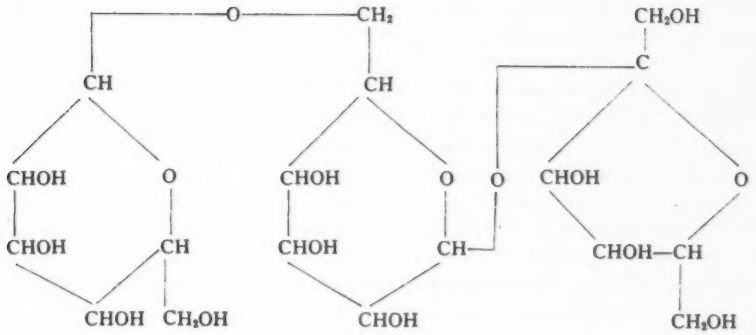
Unfortunately the rare sugars gentianose, stachyose and turanose, all of which contain a terminal fructo-furanose residue, as does sucrose, have not yet been obtainable. The action on these sugars of both *B. mesentericus* and *B. subtilis* will be studied as soon as a supply can be obtained.

The results of the above experiment indicated that there may be present in the bacterial cells an enzyme (or enzymes) capable of exerting a selective action upon the terminal fructo-furanose grouping in the sugar molecule in such a manner as to bring about the formation of a more highly polymerized product. Conclusive evidence has been obtained to show that the fructose and not the glucose portion of the sucrose molecule is involved in the polymerization (11), and this would also appear to be the case with raffinose, although it has not, as yet, been definitely established. It has been shown experimentally that the glucose part of the sucrose molecule remains unchanged (11). The levan produced by both *B. mesentericus* and *B. subtilis* from raffinose is at present under investigation.

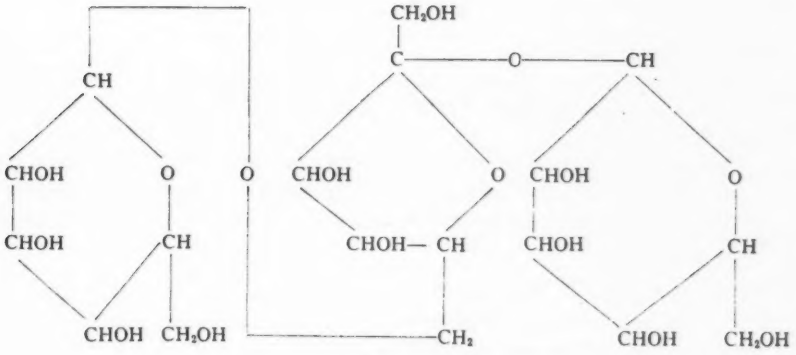
In view of the fact that the results obtained pointed to the presence of a specific levan-synthesizing enzyme in the secretions of the bacterial cells, attempts were made to demonstrate its presence. In this connection it must be recalled that Owen (20) succeeded in obtaining levan by employing the alcoholic precipitate which resulted when large masses of the cells of *Bacillus vulgatus* were triturated, water added to the triturated mass, and the resulting liquid poured into ethyl alcohol. This alcoholic precipitate was mixed with his standard nutrient sucrose solution and toluene added to prevent bacterial growth. Results obtained in these laboratories by the use of this method at once showed that trituration, followed by alcoholic precipitation of the triturated mass only destroyed a fraction of the cells, and a large percentage of the viable cells still remained in the precipitate. However, it was found that while the numbers of bacterial cells decreased greatly in the presence of toluene



SUCROSE



RAFFINOSE



MELEZITOSE

a marked increase in gum production (as judged by the amount of alcohol-precipitable substance in the solutions) was evidenced when enzyme preparations containing relatively large amounts of living cells of *B. mesentericus*, or, on the other hand, large amounts of the culture growth, were added to sterile, neutral 10% sucrose solutions. Such results, while favoring strongly the hypothesis that the levan is produced by a specific enzyme, do not prove that such an enzyme is present, for Waldschmidt-Leitz (39) states that enzymes are "formed indeed by living cells, but independent of the presence of the latter in their operation", and the above solutions contained living cells.

Owen (20) failed to obtain consistent levan formation by adding to sterile sucrose solutions bacteria-free(?) filtrates obtained from fluid cultures of *Bacillus vulgatus*. However, Beijerinck (1) appears to have obtained a preparation capable of synthesizing gum from sucrose solutions by filtering a fluid culture of *Bacillus mesentericus vulgatus* and adding alcohol to the filtrate. He believed that such preparations contained a specific levan-forming enzyme which he termed viscosaccharase. Experiments in these laboratories have substantiated the results obtained by the latter investigator. Sterile filtrates obtained by filtering a 72-hour culture of *B. mesentericus* when added in small amounts to sterile, neutral, 10% sucrose solutions brought about definite, though somewhat small, increases in the amount of substance precipitable by alcohol in the sucrose solutions. These precipitates, though not identified as levan, chemically, showed similar characteristics such as ready solubility in water to form an opalescent, greyish solution.

In summing up it would seem that a levan-synthesizing enzyme is produced by *B. mesentericus* and *B. subtilis*, although no claim can be made that a pure active preparation has, as yet, been obtained. In fact, all attempts to obtain a product which, in small amounts, can bring about the synthesis of relatively large quantities of levan from sucrose solutions have been unsuccessful. This may be due to a number of possible factors such as the great instability of the enzyme and the factors influencing its activity, such as pH, concentration of substrate, etc.

Experimental Part

Preparation of Media

Media having the following composition were employed:

Sucrose agar: sucrose, 5%; KCl, 0.5%; Na_2HPO_4, 0.2%; peptone, 0.5%; agar, 1.5%.

Sucrose broth: sucrose, 20%; KCl, 0.5%; Na_2HPO_4, 0.2%; peptone, 0.1%.

The constituents were dissolved in distilled water and the media subsequently sterilized in the final containers by autoclaving under 15 lb. steam pressure for a period of 20 min.

Preparation of Levan From Sucrose

The culture employed was isolated from commercial brown sugar by the plate method, employing sucrose as nutrient substrate. According to the systematic classification adopted by the Society of American Bacteriologists (2),

it appeared to be a typical strain of *Bacillus mesentericus* Trevisan, characterized, however, by the fact that it reduced nitrates to nitrites. Throughout the investigation the culture was transferred in sucrose broth each week, in order to maintain, at a maximum, its activity as a levan synthesizer.

Sucrose broth (20%) was sterilized in 500 cc. portions in one-litre Erlenmeyer flasks by exposing it to flowing steam at 100° C. for 30 min. on each of three successive days. The solutions were then inoculated with one loopful of a 24-hour old culture of *B. mesentericus* contained in sucrose broth, and incubated at 37.5° C. for six days. At the conclusion of this incubation period the bacterial masses were removed from the culture solutions by centrifuging at 3,000 r.p.m. for 10 min. (in a centrifuge having an effective diameter of 30 cm.), and carefully decanting the supernatant liquid. A microscopical examination of the centrifuged liquid showed it to be practically free from micro-organisms, and it was then evaporated under diminished pressure (20-30 mm.) to one-sixth its original volume. Thus in one experiment 12 litres of the centrifuged culture fluid was evaporated to approximately two litres of syrup, specific gravity = 1.232.

This solution (A) was run, at the rate of one drop per second, into cold 95% ethyl alcohol (d = 0.812) with vigorous mechanical stirring, until the precipitate began to get faintly gummy and the resulting alcoholic solution had a density of 0.846. The precipitation was arrested at this stage, the supernatant liquor (B) decanted and replaced by fresh 95% ethyl alcohol. (After standing a further 24 hr. the supernatant liquor (B) deposited an additional crop of levan which was filtered off.) When all of (A) had been treated in this manner the mixture was stirred for a further 5 to 10 min. and then allowed to stand in the refrigerator for one hour in order to settle. After the first precipitation it is advisable to decant the main solution, and only to filter the precipitated product, thus avoiding the difficulties in filtration due to the presence of a large amount of sucrose.

The white, amorphous powder obtained by this preliminary precipitation was freed from traces of sucrose and glucose by a second and third precipitation from a very concentrated aqueous solution at room temperature by 95% ethyl alcohol. It was washed on the filter with absolute ethyl alcohol and dry ether in quick succession, and dried in a vacuum desiccator over P_2O_5.

Yield of Levan as Prepared Above

Six litres of 20% sucrose broth (containing 1,200 gm. of sucrose) yielded 306 gm. of levan in the form of a white amorphous powder after two precipitations, with 95% ethyl alcohol and subsequent thorough drying. The theoretical yield is 568 gm., assuming levan to be formed entirely from the fructose portion of the sucrose molecule.

Some preliminary experiments on the precipitation of levan from aqueous solution showed that the best procedure for obtaining a powdery product, free from gum, lay in the slow addition of the aqueous levan solution, together with vigorous mechanical stirring. It is necessary to have sufficient water present in the mixture to retain the sucrose, free reducing sugars and organic acids in solution, at the same time avoiding excess, which inhibits the precipita-

tion as a fine powder and results in gum formation. If, as occasionally happens, the product should be precipitated in a slightly gummy form, it can readily be rendered friable by grinding in a mortar with absolute ethyl alcohol.

Purification of Levan by Electrodialysis

After preliminary purification by precipitation to remove reducing sugars, the sodium and potassium salts present (which accounted for an ash of 0.66%) were removed by electrodialysis in the Pauli apparatus (23).

In a typical experiment 50 gm. of levan, dissolved in 200 cc. of water, were dialysed until the electrical resistance of the centre compartment containing the levan remained constant. At the start, with an applied potential of 23 volts, the current was 0.45 ampere; as the current diminished the voltage was gradually increased so that the current remained at about 0.2 ampere during the remainder of the experiment. After a lapse of some 53 hours from the commencement, the current was 0.1 ampere with an applied potential of 224 volts. The parchment membranes were of good quality, so that none of the levan was lost. The solution showed no tendency to flocculation and no change in color of the solution was observed. The solution was then evaporated, the levan precipitated as usual, and employed in the chemical investigations (11). It was quite free from ash.

In later work an ash-free levan was obtained by the use of an enzyme preparation obtained as follows: 10% sucrose solutions were prepared by dissolving the crystalline product in distilled water, and sterilized by filtering through a Berkefeld "W", employing suction (500 mm.). One litre of the solution was placed in each of two two-litre, sterile, Erlenmeyer flasks, covered with 200 cc. of toluene and the flasks stoppered with corks. Five grams of an enzyme preparation, obtained by triturating the cells of *Bacillus mesentericus* with sterile powdered glass (see Expt. 1, later), was added to each solution, and the flasks incubated at 37.5° C., and shaken once every 24 hr. After 10 days in the incubator the solution was evaporated at 40° C. under reduced pressure (740 mm.) to a fairly thick syrup (325 cc., $d = 1.232$). The solution was now run, at the rate of one drop per second, into five litres of cold 95% ethyl alcohol. The white, amorphous powder thus obtained weighed 47.2 gm. after drying 24 hours in a vacuum desiccator over P_2O_5. It was further purified by a second and third precipitation from aqueous solution ($d = 1.232$) by 95% ethyl alcohol, washed with absolute alcohol and ether and dried in a vacuum desiccator over phosphoric anhydride. The resulting product was found to be free from ash.

The levan, prepared as above, was clarified in order to make polarimetric determinations as easy as possible. The aqueous solution was shaken with purified charcoal containing no soluble salts, and was then centrifuged at 4,000 r.p.m. (in a centrifuge having an effective diameter of 30 cm.) until the charcoal had separated. The resulting supernatant liquor was drawn off, more charcoal added and again centrifuged. The final solution was not perfectly transparent, but exhibited a pale-yellow color by transmitted light and a slight bluish-white opalescence by reflected light. The levan, after clarification

as above, was precipitated once more from ethyl alcohol, dried in a vacuum desiccator over phosphoric anhydride, and subsequently employed in the chemical investigations (11).

Origin of Levan

Experiments were next conducted with the object of determining which carbohydrates are capable of yielding levan.

50 cc. portions of a medium were prepared containing 10% carbohydrate, 0.2% Na_2HPO_4, 0.5% KCl, and 0.1% peptone, dissolved in distilled water; they were sterilized by filtering through a Berkefeld "W" filter with suction (500 mm.), and incubated 48 hours at 37.5° C. in order to control the sterility of the solutions. In this manner duplicate sets of solutions containing 10% of raffinose, melezitose, sucrose, maltose, lactose, glucose, fructose and xylose were obtained. One set was then inoculated with one loopful of a 48-hour old sucrose-broth culture of *Bacillus mesentericus*, and the other set with a similar quantity of a 48-hour old sucrose-broth culture of *Bacillus subtilis*. The cultures were then incubated at 37.5° C. for 10 days. After this period of incubation the solutions were treated in the following manner:

Each solution was filtered separately by suction through a Whatman No. 42 filter paper in order to remove the heavier bacterial membranes, the liquids made just alkaline to phenolphthalein with from 0.2 to 0.4 cc. of *N*/1 NaOH, in order to have approximately the same pH value and to facilitate precipitation, and then poured into 250 cc. of 95% ethyl alcohol contained in a weighed 500 cc. Erlenmeyer flask. In each case the alcoholic liquor was shaken thoroughly and permitted to stand half an hour only at room temperature, in

TABLE I

GUM PRODUCTION FROM VARIOUS CARBOHYDRATES BY *B. mesentericus* AND *B. subtilis*

Carbohydrate substrate	Weight of precipitate in grams	
	B. mesentericus	*B. subtilis*
Sucrose	0.6679	0.9358
Raffinose	0.3075	0.3295
Melezitose	0.0096	0.0102
Maltose	0.0118	0.0165
Lactose	0.0131	0.0164
Xylose	0.0135	0.0139
Glucose	0.0172	0.0157
Fructose	0.0140	0.0122

order to avoid crystallization of any of the unchanged sugars. During the precipitation it was noticed that heavy flakes of levan were precipitated immediately in the raffinose and sucrose solutions with both cultures, while in the case of all other sugar solutions only a moderate clouding was observed and no sign of any precipitate could be detected. After standing half an hour the supernatant alcoholic liquor was decanted from each flask and each container was then inverted and allowed to drain for one hour in an incubator at

50° C. Subsequently the flasks were permitted to stand for 24 hr. at room temperature and then weighed. The weights of the precipitates were calculated by difference and are recorded in Table I.

The results obtained in this experiment show that gum is produced in sucrose and raffinose only and in none of the other carbohydrates employed. The weights occurring in the case of the other carbohydrates may be attributed to the small quantity of alcoholic liquor, rich in sugar, adhering to the flasks.

Experiments to Substantiate the Theory of the Presence of a Specific Levan-forming Enzyme Secreted by the Bacterial Cells

Experiment 1. Petri dishes having a diameter of 10 in. were filled to a depth of approximately 0.5 cm. with sterile sucrose-agar, the surface of the agar inoculated from a fresh sucrose-broth culture of *B. mesentericus* and the plates incubated at 37.5° C. The cells were scraped from the surface of the agar with sterile glass slides after incubation for 72 hr. and 10 gm. of the moist cells triturated with an equal weight of sterile powdered glass for a half-hour, observing aseptic conditions. Sterile distilled water (50 cc.) was then added to the triturated mass, the glass allowed to settle, and the supernatant liquid carefully pipetted off and run drop by drop into 250 cc. of 95% ethyl alcohol. The resulting flocculent precipitate was permitted to settle, the supernatant alcoholic liquor decanted off and the precipitate dried by pressing between sterile filter papers*. One gram of this precipitate was introduced into a flask containing one litre of a 10% solution of sucrose in distilled water, brought to pH 7.0 by employing brom-thymol blue as indicator, and sterilized by filtering through a Berkefeld "W", with suction (500 mm.) The solution was well shaken until the precipitate had diffused thoroughly throughout the liquid and an initial bacterial count made. The solution was then covered with 10% of toluene, and incubated at 37.5° C., shaking once every 24 hours. Bacterial counts and levan determinations were made on the solution simultaneously, and at stated intervals, as follows:

The number of bacteria present was determined by the plate method, employing nutrient-agar as substrate, incubating the petri plates 24 hr. at 37.5° C., and counting the number of colonies formed. Levan was determined by removing 5 cc. of the solution from the flask under aseptic conditions, and placing the same in a weighed 100 cc. beaker. This solution was then made just alkaline to phenolphthalein with from one to two drops of *N*/1 NaOH, and 25 cc. of 95% ethyl alcohol added. The precipitate was allowed to settle by standing for 12 hr. at room temperature, the supernatant alcoholic liquid carefully decanted, the beaker placed in a vacuum desiccator over $CaCl_2$ and P_2O_5 in order to dry the precipitate thoroughly, the beaker and its contents then weighed, and the weight of the precipitate calculated by difference. The complete results of this experiment are recorded in Table II.

* *The enzyme preparation used in synthesizing the levan required for the chemical investigation (to be published later) was made in this manner.*

TABLE II

RELATION BETWEEN THE AMOUNT OF LEVAN FORMED AND THE NUMBER OF VIABLE CELLS IN PREVIOUSLY STERILIZED NEUTRAL SUCROSE SOLUTION, CONTAINING ENZYME PREPARATION AND 10% OF TOLUENE

Length of incubation period in days	Bacterial count in colonies per cc. of solution	Wt. of crude levan in gm. per 5 cc. of solution
0	22,000,000	0.0037*
7	2,370,000	0.0311
10	673,000	0.0577
14	252,000	0.0723
28	98,000	0.0210

* *Represents residue due to sucrose present in the alcoholic solution.*

These results show that increase in levan formation up to the fourteenth day of incubation, is accompanied by a decrease in the number of viable cells when such enzyme preparations are employed.

Experiment 2. The cells of *B. mesentericus* were obtained after 48 hours' growth on sucrose-agar plates following the method outlined in Expt. 1. A 10% sucrose solution was brought to pH 7.0 by employing brom-thymol blue as indicator and titrating with a few drops of *N*/10 NaOH, then sterilized by filtration through a Berkefeld "W" filter with suction (500 mm.) and 500 cc. placed in a sterile cork-stoppered one-litre flask. Two grams of the moist cells of *B. mesentericus* was added to this sucrose solution, the flask shaken thoroughly to bring about an even distribution of the cells, and a bacterial count made as in Expt. 1. The flask was then incubated at 37.5° C., after covering with 10% of toluene, and the solution was shaken once every 24 hr. Bacterial counts were made at intervals following the method employed in Expt. 1, and levan determinations were made simultaneously on 5 cc. portions of the liquid by the technique outlined in Expt. 1. The complete results of this experiment are recorded in Table III.

TABLE III

RELATION BETWEEN AMOUNT OF LEVAN FORMED AND NUMBER OF VIABLE CELLS IN STERILE, NEUTRAL SUCROSE SOLUTION CONTAINING CULTURE GROWTH AND 10% OF TOLUENE

Length of incubation period in days	Bacterial count in colonies per cc. of solution	Wt. of crude gum in gm. per 5 cc. of solution
0	9,000,000	0.0023*
5	1,030,000	0.0681
16	231,000	0.0797
34	68,000	0.0622

* *Represents residue due to sucrose present in the alcoholic solution.*

These results show that the amount of levan increases while the number of viable cells decreases up to the sixteenth day of incubation.

Experiment 3. A 72-hour old sucrose-broth culture of *B. mesentericus* (50 cc.) was filtered through a Berkefeld "W" filter employing suction (500 mm.) 5 cc. of this filtrate was found sterile after "plating" on nutrient sucrose-agar and incubating the plates at 37.5° C. for 48 hr. A quantity of a 10% solution of sucrose was brought to pH 7.0 by the addition of a few drops of *N*/10 NaOH, employing brom-thymol blue as indicator, and sterilized by filtering through a Berkefeld "W" filter, employing suction (500 mm.). Using aseptic technique, 250 cc. portions of this filtrate were transferred to sterile cork-stoppered 500 cc. Erlenmeyer flasks. Three of these, the contents of which showed no contamination after incubation for 48 hr. at 37.5° C., were inoculated with 2.5, 0.25 and 0.025 cc. respectively, of the above sterile *B. mesentericus* filtrate (immediately after filtration to avoid possible changes due to deterioration), covered with 10% of toluene to prevent contamination, and incubated at 37.5° C., shaking every 24 hr.

Examinations for the presence of crude gum (detected by precipitation with ethyl alcohol) were made on 5 cc. portions of the solutions according to the technique outlined in Expt. 1. The results are recorded in Table IV.

TABLE IV

PRODUCTION OF GUM IN STERILE SUCROSE SOLUTIONS BY STERILE FILTRATES OF A SUCROSE-BROTH CULTURE OF *B. mesentericus*

No. of cc. of culture filtrate employed as inoculum	Weight of alcoholic precipitate in gm. per 5 cc. of culture fluid after:		
	7 days	14 days	21 days
2.5 cc.	0.0071	0.0228	0.0173
0.25 cc.	0.0058	0.0192	0.0155
0.025 cc.	0.0065	0.0131	0.0071

The results of this experiment show that a definite increase in the amount of alcoholic precipitate occurs in about 14 days after the culture filtrate is added to the solution, and that this increase is followed by a slight decrease upon prolonged incubation.

Acknowledgments

The authors wish to express their thanks to Mr. R. S. Tipson for the assistance rendered by him in connection with the chemical investigations.

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OXYGEN BOMB CALORIMETER[1]

By Edgar Stansfield[2] and John W. Sutherland[3]

Abstract

This paper describes a stainless steel Scholes bomb and the modification found necessary to reduce the time for temperature equilibrium, a new calorimeter equipment, and a simplified method for correcting Beckmann thermometer readings. Notes on a vacuum cup calorimeter are also included.

The bomb calorimeter to be described includes first, a stainless steel bomb of the Scholes pattern made by G. Cussons Ltd., The Technical Works, Manchester, England, and second, a calorimeter with jacket, stirrers, etc., designed and constructed at the University of Alberta.

The Bomb

The bomb, of 660 cc. capacity, was made, by special request, of Hadfields' Era Steel, C.R.2, which is a stainless steel resistant to corrosion by either dilute nitric or dilute sulphuric acid. The base of this bomb carries the ignition posts and crucible support. The bomb cylinder forms a cover which is screwed down loosely by hand onto the base. The joint is sealed by means of a rubber ring of C-section, held tight by the pressure of the oxygen. The rubber is protected from heat by means of a continuation of the bomb walls, as shown in Fig. 1.

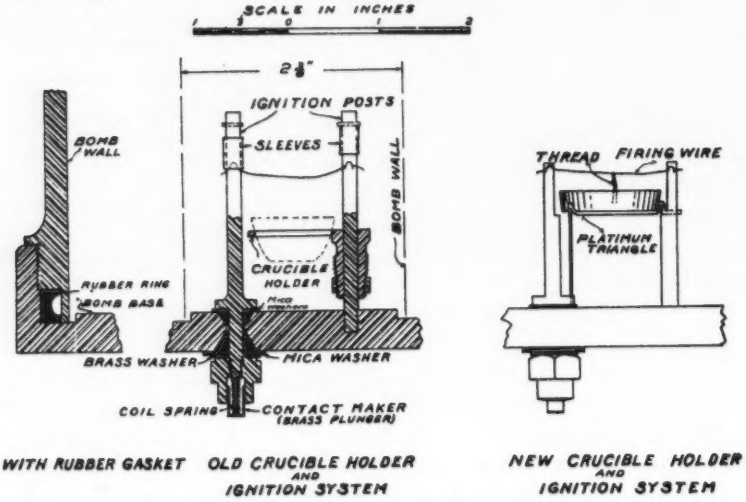

FIG. 1. *Details of Scholes bomb.*

[1] *Manuscript received October 4, 1930.*
Contribution from the Fuels Laboratories of the Research Council of Alberta, University of Alberta.
[2] *Chief chemical engineer, Research Council of Alberta.*
[3] *Chemical engineer, Research Council of Alberta.*

The oxygen is introduced through an ordinary Schrader tire valve situated in the top of the cover, but after the oxygen is introduced a metal valve with ebonite cone is also closed. As the Schrader is a non-return valve the gases at the end of a run are discharged through a separate exit valve.

The base of the bomb, as supplied, carried two stainless steel posts with slots for the ignition wire, and sleeves to lock this wire. One of these posts was carried through the base, electrically insulated from it, and attached below to a brass terminal with a spring-controlled contact pin to connect with the calorimeter cup. The other post was connected through the body of the bomb to an electric binding post on the top of the inlet valve. One of the posts also carried a heavy screw clamp and ring to carry the crucible.

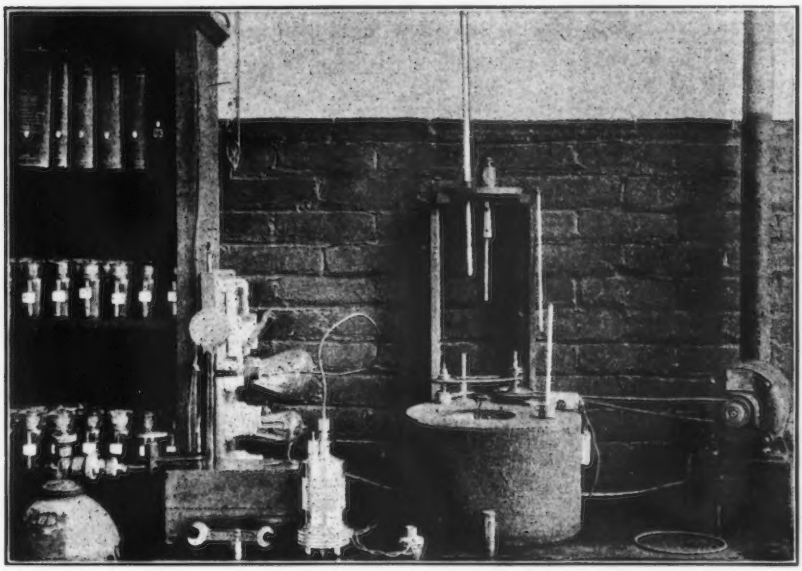

FIG. 2. *General view of apparatus with bomb removed from calorimeter.*

The steel employed has proved entirely satisfactory; after two years in use the bomb is as bright and smooth, inside and out, as when purchased. The lead gaskets used in other bombs have been found to be a recurring source of trouble, and also a cause of error when sulphur determinations are made; the rubber gasket of the Scholes bomb has proved satisfactory, and the convenience of opening and closing without a spanner is very marked. The ignition system, as such, and the valves have also proved satisfactory.

The only notable difficulty experienced was the excessive time taken for the calorimeter temperature to attain equilibrium after the charge had been fired. This was seldom less than nine minutes and often longer. The efficiency of stirring, the heat conductivity of the stainless steel, and heating of the rubber

gasket were all suspected, but none of these factors proved to be the cause. The metal around and above the crucible naturally becomes very hot during combustion, and it was found that the facility for temperature equalization between this and the rest of the system was insufficient. The difficulty was entirely overcome and the time for equilibrium reduced to five minutes by cutting down the steel ignition posts, leaving off the sleeves, and replacing the heavy crucible holder and clamp with a platinum wire triangle with two legs. The old and new systems are both shown in Fig. 1. The general appearance of the bomb may be seen in Fig. 2.

The Calorimeter

This calorimeter was designed to obtain on a small scale and at reasonable cost, as many as possible of the advantages achieved by the large constant-temperature bath and total immersion system employed by the U.S. Bureau of Mines (1).

The calorimeter chamber is of 24 gauge sheet copper, nickel-plated inside. It is hung eccentrically, as shown in Fig. 3, in a large copper bath holding 19 litres of water. The top and sides of the bath are felt-covered. The water in the bath can be heated electrically, and it is continually stirred when in use. Its temperature is observed and controlled by means of a thermometer and a bimetallic thermoregulator.

The calorimeter chamber is closed with a nickel-plated copper disc, which rests on an annular ring just below the top. The disc is held down on the ring by three light springs. This system was designed to cover the chamber with a plate in thermal contact with, and therefore approximately at the same temperature as, the water-jacketed walls of the chamber, without the complications involved by total immersion. The metal disc is hung from a transite board cover, and separated therefrom by a bakelite plate and a layer of felt to complete the thermal insulation. This transite board cover also carries the stirrer and a Beckmann thermometer. The ball bearings for the stirrer and a brass packing gland to hold the thermometer are bolted to the top of the cover. This allows the stirrer, thermometer and cover to be removed as one unit and kept on a stand behind the calorimeter ready for replacement and immediate use. The correct placing of the cover on the calorimeter is ensured by means of three studs. This cover system is shown in Fig. 2 and 4.

The calorimeter cup, also made of nickel-plated sheet copper, rests on three ebonite blocks in the bottom of the chamber. One of these blocks is wired to make the electrical connection with the cup for the firing circuit. The bomb stands inside the cup on three ebonite-capped legs. Two of the legs rest in guiding, coned supports, to permit easy yet exact placing of the bomb. Only one leg is shown in Fig. 3 to allow the insulated contact maker to be seen.

The stirrer in the calorimeter cup is shown in section in Fig. 3. It is a centrifugal stirrer of very simple pattern, consisting of a brass tube leading up into a brass cylinder of larger diameter. When the stirrer is rotated water is sucked up from near the bottom of the bomb and thrown out laterally at the

top of the bomb, by centrifugal force, from four orifices in the brass cylinder. An ebonite plug is used to connect the stirrer to its steel shaft, and yet prevent heat losses. To further guard against heat losses the shaft is surrounded by a glass tube between the ebonite plug and the top of the cover. The glass tube just passes freely through the cover. A 0.25-h.p. motor is used to drive pulleys on a jack shaft on the top of the calorimeter. The jacket stirrer and the calorimeter stirrer are driven from these by means of rubber belts. A general plan of the cover is shown, half scale, in one corner of Fig. 3.

Operation

A constant-temperature room is not available for this work so the jacket-water temperature is maintained distinctly above the highest room temperature

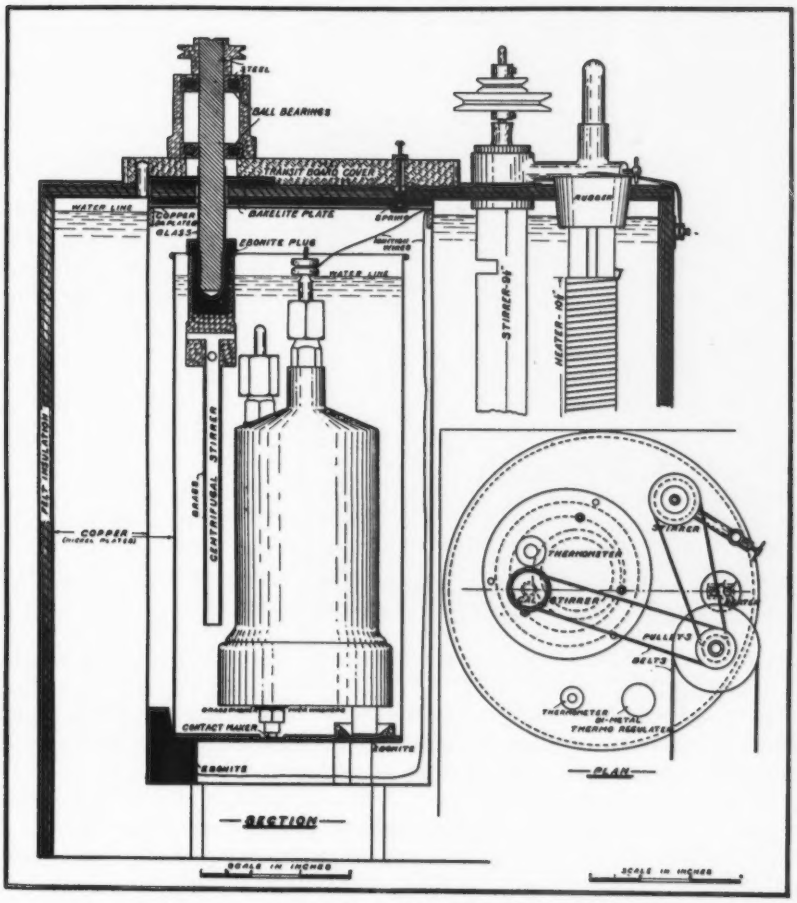

FIG. 3. *Calorimeter with Scholes bomb for solid and liquid fuel calorimetry.*

anticipated at the current time of the year, commonly at 26-28° C. If necessary a small current is kept running through the jacket heater to prevent rapid cooling during a run, but the thermoregulator is always cut out of action before beginning a run. The calorimeter water is cooled between successive runs to about 1.5° C. below the jacket temperature, and the weight of water plus calorimeter cup adjusted to balance a prepared counterpoise. The weight of water taken is 1,622 gm. and the water equivalent of the whole system is 2,047 gm. The weight of coal or other fuel burned is adjusted to give an estimated rise of about 2.5° C.

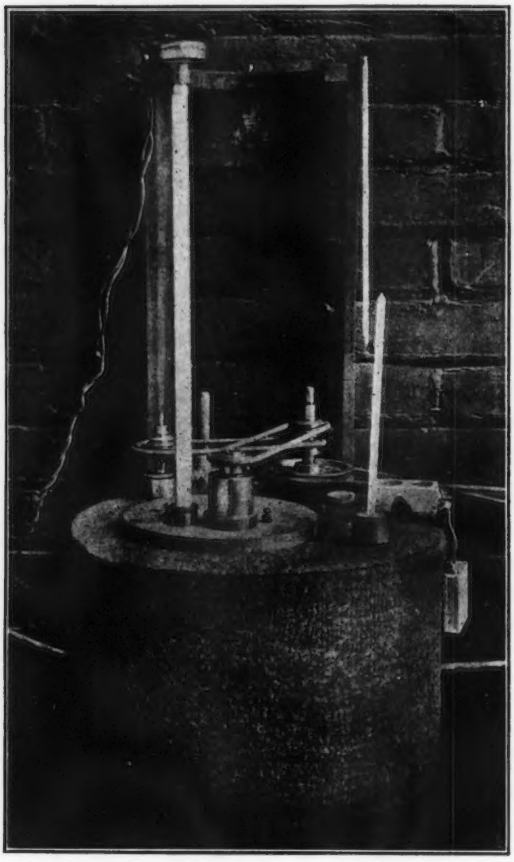

FIG. 4. *View of the apparatus showing cover, thermometers, thermoregulator and stirring arrangement operatively assembled.*

The thermal insulation of this calorimeter is found to be as good as that of the vacuum cup calorimeter previously employed. The change in rate of cooling per minute, per degree of rise in calorimeter temperature, that is, the "cooling factor", averages about 26 ten-thousandths of a degree.

The time taken for a run is reasonably short; an experienced operator during steady running can complete one every 40 min. This timing can be maintained when the same operator does all the work, including titration of bomb rinsings and calculation of the result. Times as low as 33 min. have been recorded in ordinary work.

Beckmann Thermometer Corrections

The calculation of results has been simplified by including in the setting factor the correction for emergent stem. The setting factor for a Beckmann thermometer varies with the setting temperature. These values are shown

in tabular form in the Bureau of Standards certificate for the thermometer. For five-degree range thermometers of Jena 16^{III} glass, at ordinary room temperatures, these values are represented by the equation

$$f = 1.000 + 0.0003\ (S\text{-}20)$$

where f = the setting factor, and S the setting temperature, in degrees Centigrade. The correction to be added for emergent stem is given by the equation

$$\text{Correction} = Kd\ (S + T_1 + T_2 - t)$$

where K is the factor for relative expansion of glass and mercury (0.000155), T_1 and T_2 are the initial and final readings (corrected for irregularity of bore), S as before, the setting temperature of the thermometer, t the temperature of the emergent stem and $d = T_2 - T_1$.

The corrected rise, in degrees Centigrade, equals fd + emergent stem correction, or from the above $= d[f + K(S + T_1 + T_2 - t)]$, or $= dF$, where F is the new correction factor to include both setting and emergent stem corrections.

In ordinary operations no serious error is introduced by assuming an average value of 5 for $T_1 + T_2$. If the known numerical values are inserted the value of F becomes $n + 0.9948 + 0.000455\text{S} - 0.000155t$, where n equals a small adjustment for the particular thermometer in question. Solving for n for a particular thermometer, with stated values of $S = 10°$ and 20°, an average value of -0.0008 was obtained, so that the equation became

$$F = 0.994 + 0.000455\text{S} - 0.000155t.$$

Values of F for different setting temperatures from 10° to 30° C. and for room temperatures (emergent stem temperatures) from 15° to 30° C. are plotted in Fig. 5. From this it can be seen, for example, that at a setting temperature of 22° C. and an emergent stem temperature of 20° C. the correction factor by which the rise in temperature ($T_2 - T_1$) must be multiplied is 1.001. For any setting temperature the correction factor can be tabulated for different room temperatures.

Notes on a Vacuum Cup Calorimeter

A vacuum cup calorimeter, purchased in 1921, was in constant use in this laboratory for nearly seven years. There were many unsatisfactory features about this equipment, but the following notes only refer to the use of a vacuum cup.

Twice during this period the cup was left in apparently good condition after use in the afternoon, and found shattered next morning. No cup was broken in actual use.

When the calorimeter was first put into use its water equivalent was carefully determined, and it was assumed that this value would remain unchanged. This apparently was not the case. Unfortunately, however, only one set of determinations of the water equivalent was made while the first cup was in use.

The change in water equivalent found when the second cup was put in use was greater than was expected from any probable difference in the weights of the cups. The first cup was never weighed. The third cup was 102 gm. heavier than the second cup, yet the water equivalent of the system with this cup was about 13 gm. lower than the average value with the second cup.

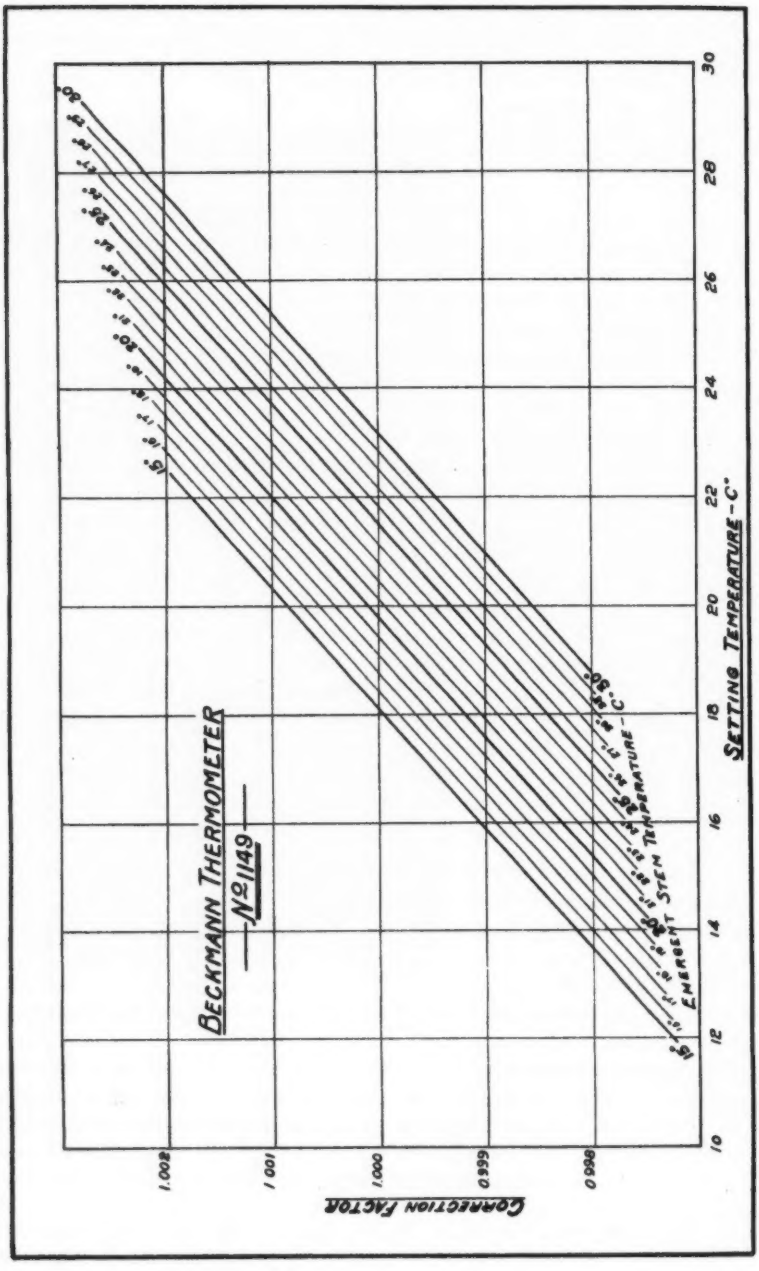

FIG. 5. *Correction factor chart.*

There was a notable drop in the determined values of the water equivalent of the system during the life of the second cup, but as the low values were only based on four runs—a series interrupted by the collapse of the cup—it is not safe to place much emphasis on this point. There is, however, strong evidence that the cups changed, probably due to slow failure of vacuum, with a corresponding change in the water equivalent of the system. The third cup was stored in reserve in the laboratory for 18 months and it is believed that the vacuum was already poor when it was put into commission. This cup is still in existence, seven years after its purchase.

The most notable variant in the calorimeter system is a factor we call the cooling factor, which is the change in rate of cooling per minute, per degree of rise in calorimeter temperature. It is expressed below in ten-thousandths of a Centigrade degree. Thus if, during the steady period before firing the average temperature as shown by a Beckmann thermometer is 1.053° C. and the rate of cooling is −0.0030° C. per minute, and if, during the steady period attained after the maximum temperature had been reached the average temperature is 3.617° C. and the rate of cooling is +0.0012° C. per minute, then the change of temperature is 2.564° C. and the rate of cooling has changed in consequence from −30 to +12 ten-thousandths of a degree per minute. The cooling factor is therefore 42 divided by 2.56, that is 16. This factor varies considerably from run to run owing to changes of room temperatures during the run, but the average value from day to day is usually very steady. In order to remove casual variations in this factor, the values found have been averaged over two-month periods. These values are based on more than 1,700 calorimeter runs distributed over nearly seven years. The cooling factor with the first cup began at 17, fell gradually to 10, and rose again to 17. With the second cup it began at about 20 and rose slowly to 29. With the third cup it remained fairly steady at slightly over 30. A surprising feature is that these values form a fairly regular curve, regardless of the change of cup.

The changes in the cooling factor cannot be correlated with the seasons of the year. One possibility considered was that they were due to the steady wearing away of the edges and packing down of the felt lining in the cover. This lining was replaced with new, close fitting felt, but no decrease in the cooling factor could be noted.

A significant fact ultimately noted was that the increase in cooling factor appeared to be associated with a decrease in water equivalent. It might be noted at this point that the change in water equivalent could not be explained as caused by combustible impurity in the oxygen supply.

It is possible that the same vacuum cup calorimeter system may behave in two different ways. It may be in a condition in which the heat capacity of the inside wall of the vacuum cup is included in the determined water equivalent of the system, and the heat which escapes from the inside wall to the outer wall, across the partial vacuum or by conduction along the glass, is included in the heat losses responsible for the cooling factor. Or it may be in a condition in which the water equivalent of no part of the cup is included in the determined water equivalent, and any heat which escapes from the calorimeter jacket to

the cup is included in the cooling factor. It is suggested that a change from the first condition to the second might be caused by failure of vacuum in the cup and would be accompanied by increase of cooling factor and decrease in water equivalent.

This theory was not proved as it was simpler and better to replace the vacuum cup calorimeter with a different type of calorimeter. These notes are published with the hope that other laboratories having data on this problem will confirm or disprove the theory. It appears that the water equivalent of a vacuum cup calorimeter should be carefully watched, and that the cooling correction cannot be ignored.

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THE MAINTENANCE OF A STANDARD OF ELECTROMOTIVE FORCE: NOTES ON STANDARD WESTON CELLS[1]

By A. Norman Shaw[2] and H. E. Reilley[3]

Abstract

A detailed procedure for the maintenance of a standard of voltage to within one or two parts in a million is described.

In so far as these investigations have proceeded, neutral saturated cells have been found to be superior to acid cells as independent standards for a period of many years, though the latter are preferable for purposes of ordinary precision or shorter periods. The recommended code of procedure is briefly as follows: a number of cells should be constructed according to standard specifications with the new requirements of uniformity of container and speed of preparation, and observations made upon them every few days for a period of three months. The differences in electromotive force (at constant temperature) should be determined between each cell in the group and any one of them chosen arbitrarily as reference cell, and certain new selection and rejection rules applied. In accordance with these rules a cell should be rejected: (a) if its deviation from the mean electromotive force of the group has increased or decreased by 10 microvolts or more during the preceding two weeks; or (b) if it differs in electromotive force from the mean of the group by more than $10+d$ microvolts where d is the mean deviation of the cells of the group. If d exceeds 20 microvolts the entire group should be considered untrustworthy. The selected cells should be observed for three additional months, the rejection rules again applied and if a specified proportion survive elimination, the initial reference mean of the laboratory may be established.

At intervals of several months additional groups of cells, neutral and acid, should be constructed and exchanges made with laboratories possessing cells of known characteristics. Analysis of the resulting observations determines: (a) the constants in the aging* equation for the reference batch, and (b) the difference between the initial reference mean of the laboratory and the estimated value of the international reference mean.

Examples of the analysis of cell observations are given, illustrating the establishment of the initial reference mean, the recapture of this value when the aging coefficients are known, and the preliminary determination of the aging equation for a given group of cells. The use of the aging equation is found to be the essential feature in the attainment of increased precision.

A summary of data on standard cells is included.

Introduction

This article is written to provide detailed consecutive directions which it is hoped will enable any laboratory to maintain a reference standard of voltage with the new increased precision recently developed at McGill University. A summary of the contributions from this laboratory on Weston standard cells, with notes on supplementary data arising from recent work, is also included.

The main records of data and analyses of cell observations on which this article is based, are available in a number of papers (2, 3, 7, 8, 9) and the reader is referred to these accounts for further information about the ordinary technique, and for the verification of the experimental basis and theory on which the new procedures and directions rest.

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* *The authors wish it known that they recommend and use the spelling "ageing" rather than the form "aging" adopted by the Journal.*

The main uses to which these procedures may be applied are:

(a) To enable a reference standard of electromotive force to be maintained with a precision of a few parts in a million, by means of a relatively small number of cells and modest equipment. Considerable increase in precision, and at the same time decrease in both cost and extent of equipment are made possible.

(b) To enable the recapture of a former "reference mean" by the aid of old cells, thus frequently making possible the recalculation and correction of important measurements made at different times and places.

The Construction and Selection of a Reference Standard of Electromotive Force: the Determination of the Reference Means

Although modern acid cells may probably be substituted later, on the ground of their slower aging, it is not yet advisable to use them in small numbers as an independent standard for a period of many years. It remains for them to be subjected to a more extended analysis with regard to aging, in order that old acid cells may lead to accurate comparisons (to within a few parts in a million) between measurements made on dates separated by many years. The remarkable steadiness of acid cells for three or four years is still balanced unfavorably by the high percentage which become erratic or useless sooner than do the average neutral saturated cells of high grade. In this case slow aging (*i.e.*, decrease in e.m.f. with time) is apparently not synonymous with longevity. There is, however, every reason to expect that further study should lead to the possibility of their superiority as primary reference standards, and at present for purposes of ordinary precision or for periods of a few years they may already be deemed superior. In the meantime if it is desired to survey measurements over many years with the high precision recently developed, and to rely with assurance on the maintenance of the standard, we are on more certain ground if use is made of neutral saturated cells constructed according to the well-known specifications of Wolff and Waters (11), or F. E. Smith (10), or as modified in this laboratory (3,8).

The procedure may be summarized as follows:

(a) A thermostat is constructed capable of being maintained at constant temperature for several days continuously to within 0.01°. Directions for the construction of such a thermostat are now available from many sources, but a previous paper (6) by one of the authors may be consulted to advantage.

(b) Taking particular care about insulation, there is assembled the ordinary equipment for making cell comparisons by measuring the difference in e.m.f. of two cells, opposing one another, on a simple potentiometer wire along which is a fall in potential of one microvolt per millimetre. In this way with relatively crude apparatus the differences between cells may be measured to within a few parts in a thousand. If a given difference is known to be 10 microvolts, an uncertainty of even 5% in this would not matter; so the difficult and expensive technique for potentiometer measurement of the full voltage to within one

part in a million (sometimes unnecessarily attempted) is by this measurement of differences usually avoidable. It should be noted that although a reference value may now be maintained or recaptured to within a few parts in a million, this question of comparison and reproduction remains distinct from the question of absolute values which requires more elaborate measurement and so far cannot be determined with one-tenth of the above accuracy. If it is required to deal with the full voltage of a single cell with the utmost precision the Kelvin-Varley slide potentiometer, specially calibrated, is strongly recommended (5). With this special calibration, voltages differing greatly in magnitude can be compared in cases where the simple wire potentiometer would be inadequate.

(c) At least 15 normal neutral cells are constructed according to standard specifications or with modifications the effects of which are known exactly.

Each cell should be in the same kind of container, and constructed at approximately the same speed of preparation. There should not be more than three or four cells in the group made from the same supply of ingredients. If any of these cells have to be rejected, as is usual, to fulfil the instructions in (d), another batch should be constructed to meet the requirements if there are fewer than 10 acceptable cells left.

Although five or six cells may often be adequate, it is generally advisable to maintain at least 10 cells that fulfil the final requirements, although it has been found that a batch much larger will not in general lead to any further increase in precision, under the existing methods of measurement and interpretation.

If an apparently good supply of old cells is available, it is necessary that their age should be known, that the details of their construction can be ascertained, that they should not have experienced any rough or unknown treatment, and that they should have approximately the same rates of aging. Otherwise, and usually, new cells must be made and kept inviolate if the desired precision is to be obtained, but the ultimate precision will not be available until after the cells are relatively old.

(d) For three months periodic examinations are made of the differences in electromotive force (at constant temperature) between each cell in the group and any one of them chosen arbitrarily, as reference cell. Any cell is rejected; (i) if it has increased or decreased its deviation from the mean e.m.f. of the batch by 10 microvolts or more during the preceding two weeks; or (ii) if it differs in e.m.f. from the mean of the group by more than $10+d$ microvolts, where d is the mean deviation of the cells of the batch. If d exceeds 20 microvolts the whole batch is not trustworthy. (In the case of certified cells of known history and satisfactory requirements which are over five years old, the limit $20+d$ may usually be used safely.)

(e) Observations on the e.m.f. of each cell (in terms of the mean of the group as before) should now be made from time to time during a second period of three months. At the end of this period, if the cells still maintain the selection requirements for deviations, the mean e.m.f. of the batch is taken as the preliminary *permanent* or *initial* reference mean for the laboratory.

(f) In order to be able to recapture this initial reference mean at any future date, it is now necessary to determine the aging coefficients or constants in the equation

$$E_M = A + B \log (M + \tau) = \bar{E} - \bar{E}_i$$

where E_M is the difference in e.m.f. from the initial reference mean at the age M months, measured from the date of construction, and A, B and τ are constants; $\bar{E}$ is the total e.m.f. and $\bar{E}_i$ the e.m.f. corresponding to the initial reference mean. If logarithms to the base 10 are used it may be assumed that $B = -39.4$ for all neutral cells of standard specifications. The experimental and theoretical basis for this equation has been given in detail previously by Shaw, Reilley and Clark (9).

The simplest method of finding the values of A and τ would be to transport three or more cells carefully by hand to a laboratory where cells with known aging coefficients are available. Exchanges of this kind should be repeated every six months for about two years, at the end of which the constants could be determined with maximum precision from these direct comparisons. The value of A obtained in this way would however differ from the desired value of A by an amount equal to the difference between the initial reference means in use respectively at the two laboratories; when this is obtained, as below, the determination is complete.

This does not mean that a wait of several years is necessary before important measurements with the cells can be made. Once the aging formula is obtained (or at any time when it is corrected as a result of longer records), all previous measurements involving the use of the cells can be recaptured and corrected.

However, it may be desired to establish a cell laboratory in an entirely independent manner. In this case it would be necessary to manufacture at least two new groups of cells at intervals of six months or a year, and repeat on a small scale the analysis employed in the cell laboratory at McGill University; but as a result of the knowledge of the constant B and the procedure, in advance, the records of comparisons between old and new cells for one or two years would be adequate.

There is an alternative procedure which is satisfactory for all ordinary cases requiring high precision. It is assumed that an accuracy of a few parts in 100,000 will be adequate at first, and then at a later date the results can be corrected as a result of the future records which will render the precision of a few parts in a million attainable. A batch of modern acid cells is made and at the age of a few months compared with the standard neutral batch; the assumption is made that the mean of the acid cells remains constant for many months, and thus by comparison, it is possible to deduce approximate values of A and τ which will suffice until records for several years are available.

It is clear that comparisons with other laboratories possessing cells of known aging characteristics should be the simplest and quickest course, and it is therefore recommended that the large standards laboratories should henceforth be in a position to furnish this information as to A and τ, which will enable future readings of cells (submitted to them for a few months for certification) to be obtained with higher accuracy as well as their values merely at the time of comparison.

(g) In addition to the knowledge of the aging coefficients, it is often also necessary to know the difference between the initial reference mean at the given laboratory and the international reference mean, or that of some other laboratory. This can be obtained approximately as a result of exchanges with other laboratories, or more accurately after observations over a period of years in the manner described in detail in the paper quoted. The latter course is tedious, and the approximate result of one comparison of reference means between two laboratories cannot always be trusted to closer than a few parts in 100,000, although subsequent comparisons will progressively improve the limits of accuracy.

Here again the detailed knowledge of the aging constants of various types of cells at a central laboratory would be of great value and would not only render exchanges unnecessary, but would facilitate the attainment of higher precision than would be possible with ordinary exchanges. The procedure should be merely to inquire from another laboratory what value of the constant A is found with cells having a given value of τ equal to that of the reference batch at the first laboratory; the difference between the respective values of the constant A obtained at each laboratory (for its own cells having the same given value of τ), should in general give the difference between their permanent reference means to within a few parts in a million, provided that it is known that the ingredients and specifications are in every way identical. As this is occasionally uncertain, (for example slight differences in the amalgam, or certain impurities will affect the value of A without changing that of τ), the simplest check is obtained by comparing the values of acid cells expressed in terms of the reference means for neutral cells, as reported independently at each laboratory when using its own cells. If the acid cells are uniform in e.m.f., and of the same age at each laboratory, (and if they include cells made from ingredients from various sources), it may be assumed that they will differ by less than one part in 100,000. With the aid of other comparisons with different batches and over extended periods of time, (each laboratory reporting on its own cells without exchange), this calculated comparison of reference means could be brought nearly to the same high precision with which each laboratory can recapture its own initial reference mean.

In regard to carrying out these directions, the reminder that the greatest care is necessary in the preliminary chemical work with the ingredients cannot be emphasized too strongly. As this branch of the work is discussed in detail in the publications of the great standards laboratories it has been passed over lightly in reports by the authors, but the investigator may be warned that without the most meticulous attention to specifications it will be impossible to manufacture cells of sufficiently high grade to meet the preliminary selection and rejection rules that have been found necessary if the new high precision is to be obtained. All these directions are solely for that end; if an assured precision of one part in 10,000, or even a little better, is all that is wanted, it is only necessary to purchase a few cheap certified cells from time to time, and avoid the care of a cell laboratory of the improved calibre which the authors have been endeavoring to show is possible.

Examples of the Analysis of Cell Observations

(*a*) *Illustration of the Establishment of an Initial Reference Mean*

It is assumed: (i) that the following readings have been taken with the selected cells at the age of six months, the figures representing the differences in millionths of a volt of the e.m.f. of each cell from that of the cell having the lowest e.m.f. of the group; (ii) that previous observations show the desired constancy during the preceding two weeks, and (iii) that the customary care has been taken in regard to the proper operation of the thermostat and the avoidance of any drawing of appreciable current during the potentiometer comparisons. Each reading has also been repeated in order to eliminate fluctuations due to temporary changes.

TABLE I

SAMPLE SET OF READINGS AT AGE SIX MONTHS

Cell No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Mean reading of batch	Mean deviation
Reading in microvolts	+0	+41	+66	+68	+68	+101	+65	+58	+68	+47	+123	+66	+68	+67	+69	+65	—
Deviation from mean in microvolts	65	24	1	3	3	36	0	7	3	18	58	1	3	2	4	—	15

It will be seen that cells No. 1, 6 and 11 have a deviation greater than $10+d$, which is 25 in this case. These cells are therefore rejected, and the record with changed deviations is shown in Table II.

TABLE II

SAMPLE SET CORRECTED BY THE "$10+d$" RULE

Cell No.	2	3	4	5	7	8	9	10	12	13	14	15	Mean reading of batch	Mean deviation
Reading in microvolts	+41	+66	+68	+68	+65	+58	+68	+47	+66	+68	+67	+69	+63	—
Deviation from mean in microvolts	22	3	5	5	2	5	5	16	3	5	4	6	—	7

It is now obvious that the table still fails to fulfil the requirements, and repeating the above process, cell No. 2 must be rejected. It is unnecessary to show the tables for the effect of this correction as it can be seen by inspection that cell No. 10 will next come into the category of undesirables. (In some cases the rejection of cells can be done easily by inspection, as would have been possible in this illustrative set of very good cells, but more often it is advisable to proceed as indicated.) The results of this second correction are shown in Table III.

TABLE III

SAMPLE SET CORRECTED A SECOND TIME

Cell No.	3	4	5	7	8	9	12	13	14	15	Mean reading of batch	Mean deviation
Reading in microvolts	+66	+68	+68	+65	+58	+68	+66	+68	+67	+69	+66	—
Deviation from mean in microvolts	0	2	2	1	8	2	0	2	1	3	—	2

The mean reading for this batch on the given date of observation may now be taken as the initial reference mean. The readings of these cells, and indeed all other readings which have been referred to cell No. 1, may now be expressed in terms of the initial reference mean by subtracting the final mean reading obtained, namely 66 in this case. The final record is shown in Table IV.

TABLE IV

SAMPLE SET EXPRESSED IN TERMS OF INITIAL REFERENCE MEAN

Cell No.	3	4	5	7	8	9	12	13	14	15	1	2	6	10	11
Difference from initial reference mean in microvolts	+0	+2	+2	−1	−8	+2	+0	+2	+1	+3	−66	−25	+35	−19	+57

Comparisons of other voltages with any of these cells at the time the above observations are made may now be expressed in terms of the initial reference mean. To capture this initial mean on a future occasion from the readings then obtained requires the use of the aging equation.

(*b*) *Illustration of the Recapture of an Initial Reference Mean When the Aging Coefficients are Known.*

It is assumed that the aging equation for this batch is given by

$$\overline{E}-\overline{E}_i=E_M=35.6-39.4\log_{10}(M+2.0)$$

where $E_6=0$, and that on a date two years and ten months after the above readings were taken, (that is, when the cells were three years and four months old) the following comparisons are obtained, with reference to cell No. 3 which had been equal in voltage to the initial reference mean at the earlier date. The application of the equation is not always quite as simple as might at first be expected.

TABLE V

SAMPLE SET OF READINGS AT AGE FORTY MONTHS

Cell No.	3	4	5	7	8	9	12	13	14	15	1	2	6	10	11
Difference from cell No. 3 at age 40 months in microvolts	+0	+28	+22	+18	−13	+24	+22	+27	+25	+35	−41	+0	+22	−8	+31

It is apparent that cells No. 3 and 8 have altered relatively to the rest of the selected part of the batch, although they have been found to be sufficiently steady during the preceding two weeks; and it might appear at first sight as

if the selection requirements would reduce the group unduly and could not therefore be fulfilled without recourse to other cells. However, the formerly rejected cells, at the right, are available for estimating the change and may be used as in Table VI. This situation is characteristic of many that arise during the history of a batch of cells, and is worthy of detailed consideration as a useful example of procedure when the number of cells is limited.

TABLE VI

ANALYSIS ILLUSTRATING RECAPTURE OF INITIAL REFERENCE MEAN

	Cell No.	3	4	5	7	8	9	12	13	14	15	1	2	6	10	11	Mean
I	Difference from initial reference mean at age 6 months (as above)	+0	+2	+2	−1	−8	+2	+0	+2	+1	+3	−66	−25	+35	−19	+57	—
II	Difference from cell No. 3 at age 40 months	+0	+28	+22	+18	−14	+24	+22	+27	+25	+35	−41	+0	+22	−8	+31	—
III	Row II minus row I	+0	+26	+20	+19	−6	+22	+22	+25	+24	+32	+25	+25	−13	+11	−26	+14
IV	Deviation from mean difference, 14	14	12	6	5	20	8	8	11	10	18	11	11	27	3	40	14
V	Row II minus row I, with cells No. 6 and 11 rejected (10+d rule)	+0	+26	+20	+19	−6	+22	+22	+25	+24	+32	+25	+25	—	+11	—	+19
VI	Deviation from mean difference, 19	19	7	1	0	25	3	3	6	5	13	6	6	—	8	—	8
VII	Row II minus row I with cells No. 6, 11, 3 and 8 rejected (10+d rule)	—	+26	+20	+19	—	+22	+22	+25	+24	+32	+25	+25	—	+11	—	+23*
VIII	Deviation from mean difference, 23	—	3	3	4	—	1	1	2	1	9	2	2	—	12	—	4
IX	Row II for age 40 months with 23* subtracted from each figure	−23	+5	−1	−5	−37	+1	−1	+4	+2	+12	−64	−23	−1	−31	+8	—
X	Row IX minus the aging correction, 28, giving the values at age 40 months in terms of initial reference mean	−51	−23	−29	−33	−65	−27	−29	−24	−26	−16	−92	−51	−29	−59	−20	—

NOTE:—*Results are given in microvolts.*
**Note carefully the source of the figure 23 in this case.*

This table is self-explanatory apart from the last row, and it should be noted that without the aging correction or other cells, it would be necessary to stop at row IX, and thus make an average error of 28 microvolts. (It could, of course, be assumed that the aging was about six microvolts per annum, formerly accepted as the only estimate available, but this figure was obtained as a result of observing cells with slow initial aging, that is, having high values of the constant τ; this procedure would lead to a correction of only 17 microvolts leaving therefore a net error of 11 microvolts in the estimate.)

Assuming that the aging equation for the batch is as given above, it will be seen that

$$E_{40} - E_6 = 35.6 - 39.4 \log_{10}(40+2) - 0$$
$$= -28.4 \text{ microvolts.}$$

As the analysis in Table VI gives values in row IX which would correspond to no average change in the picked cells, it is therefore to this row that the aging correction should be applied.

It is of further value as a direction in procedure, to note that a close approximation to the results of Table VI can be obtained, without any knowledge of the earlier readings, provided that the aging equation is known. The problem now is, given Table V and the aging equation only, to express the readings in terms of an initial reference mean assumed to be the value of the selected part of the group at the age of six months. In Table VII the procedure is apparent, leading to results which differ from those of Table VI by only two microvolts. This small difference arises from the inability in this case to determine past departures from the selected category. In ordinary practice it is rare for a discrepancy of more than four or five microvolts to arise in this way, and it is found that with full records, or with the aid of auxiliary batches, such differences may usually be eliminated by averaging.

TABLE VII

ALTERNATIVE ANALYSIS ILLUSTRATING RECAPTURE OF INITIAL REFERENCE MEAN

	Cell No.	3	4	5	7	8	9	12	13	14	15	1	2	6	10	11	Mean reading of batch	Mean deviation
I	Difference from cell No. 3 at age 40 months	+0	+28	+22	+18	−14	+24	+22	+27	+25	+35	−41	+0	+22	−8	+31	+13	—
II	Deviation from mean	13	15	9	5	27	11	9	14	12	22	54	13	9	21	18	—	17
III	Row I with cells No. 3, 8, 1, 2 and 10 rejected*	—	+28	+22	+18	—	+24	+22	+27	+25	+35	—	—	+22	—	+31	+25	—
IV	Deviation from mean	—	3	3	7	—	1	3	2	0	10	—	—	3	—	6	—	4
V	Readings referred to mean of selected batch	−25	+3	−3	−7	−39	−1	−3	+2	+0	+10	−66	−25	−3	−33	+6	—	—
VI	Readings referred to initial reference mean by aging correction, −28	−53	−25	−31	−35	−67	−29	−31	−26	−28	−18	−94	−53	−31	−61	−22	—	—

NOTE:—*Results are given in microvolts.*

**If the 10+d rule is applied once, cells No. 8 and 1 go; if applied again, cell No. 10 goes; and finally cells No. 3 and 2 go; this leaves a group of ten fulfilling the requirements of the rule.*

(c) *Illustration of the Preliminary Determination of the Aging Equation for a Given Batch of Cells*

Obviously the final and essential requirement in the last illustration is the knowledge of the aging equation, and the most accurate evaluation of this can only be obtained with the aid of a series of comparisons with a laboratory equipped with cells of known aging characteristics, or by means of a long period of investigation and analysis as described in a previous paper (9). A preliminary or tentative evaluation may, however, be made in several ways leading

to a precision still of a high order. One method requires an exchange with a standards laboratory, (not necessarily equipped to report on the values of τ), and the construction of a few acid cells according to standard specifications.

As an example of this method applied to the group discussed, let it be assumed that at the time the observations in Table IV were made, four cells A, B, C and D were received from a standards laboratory and later returned, with four others, say No. 1, 2, 3 and 4. In Table VIII, the simple record and analysis for such a comparison is shown. X, Y and Z are acid cells to be used subsequently.

TABLE VIII

ANALYSIS OF EXCHANGES

Cell	Readings at the other laboratory in terms of its reference mean			Readings at the home laboratory in terms of its initial reference mean			Estimates of initial reference mean in terms of reference mean of other laboratory	Set No. 2 referred to the other reference mean by subtracting 7
	Set No. 1 before transport to home laboratory	Set No. 3 after return transport to other laboratory	Mean	Set No. 2 after receipt of A, B, C, D	Set No. 4 after return of cells No. 1, 2, 3 and 4 to home laboratory	Mean		
A	+ 2	+ 6	+ 4	+13	—	—	− 9	+ 6
B	−20	−18	−19	−10	—	—	− 9	−17
C	−18	−19	−18	−14	—	—	− 4	−21
D	−12	− 9	−10	− 4	—	—	− 6	−11
Mean of A, B, C, D	−12	−10	−11	− 4	—	—	− 7	−11
No. 1	—	−75	—	−66	−69	−68	− 7	−73
No. 2	—	−33	—	−25	−23	−24	− 9	−32
No. 3	—	− 4	—	+ 0	+ 3	+ 2	− 6	− 7
No. 4	—	− 4	—	+ 2	+ 4	+ 3	− 7	− 5
Mean of No. 1, 2, 3, 4	—	−29	—	−22	−21	−22	− 7	−29
X	—	—	—	−57	−55	−56	—	−64
Y	—	—	—	−59	−59	−59	—	−66
Z	—	—	—	−60	−58	−59	—	−67

NOTE:—*Results are given in microvolts.*

This table shows how in this case it would be found that the initial reference mean differs from that of the standards laboratory by about seven microvolts. The values of the acid cells check satisfactorily with this in the order of magnitude, suggesting that the reference mean of the other laboratory does not differ more than a few microvolts from the international mean value. However, the small number of cells involved, and the assumption of constancy during transportation may easily, in practice, lead to larger errors. In addition to this the reference means at standards laboratories have not in the past been maintained with the high constancy which now seems possible, and sometimes they probably fluctuated through a range of several microvolts in a short time. All that can be considered as certain from Table VIII, is that the various cells, neutral and acid, are shown to be satisfactory and worthy of use for the preliminary estimate of the aging equation.

It remains to use the acid cells for an approximate determination of the constants in the equation

$$E_M = A - 39.4 \log_{10}(M + \tau)$$

It is apparent that

$$E_x - E_y = -39.4 \log_{10}\left(\frac{x+\tau}{y+\tau}\right)$$

and a solution of τ can be obtained provided that there are no arithmetical limitations arising from the logarithmic nature of the formula. The smaller the value of τ, the less its importance, and the greater the difference between x and y the more accurately may τ be determined.

It is assumed that the acid cells quoted in Table VIII are observed from time to time, and that readings taken are compared at times corresponding to Table IV (the same as Table VIII) and Table VII respectively. In Table IX an analysis of such observations is given, showing a simple method of obtaining the aging on the assumption that the acid cells age only about one microvolt per annum. Only three good acid cells are shown in the table, but it is wiser to use a larger batch in case of divergences.

TABLE IX

PRELIMINARY ANALYSIS IN THE DETERMINATION OF THE AGING COEFFICIENTS

Age of neutrals in months	*6	7	8	9	10	20	38	39	**40	41	42
Mean of selected batch taken as	+0	+0	+0	+0	+0	+0	+0	+0	+0	+0	+0
Sample readings obtained as in row 5, Table VII											
Cell No. 3	+0	+0	+2	+3	+1	−20	−24	−22	−25	−22	−22
Cell No. 4	+2	+0	+4	+3	−6	+2	+2	+3	+3	+2	+5
Cell No. 5	+2	+2	+4	+2	+0	−2	−2	−4	−3	−1	−1
Acid cells in terms of mean of neutral batch											
Cell X	−57	−56	−54	−51	−50	−40	−32	−31	−31	−31	−30
Cell Y	−59	−55	−55	−54	−54	−43	−33	−32	−32	−32	−33
Cell Z	−60	−56	−55	−53	−52	−46	−33	−34	−35	−31	−33
Mean of cells X, Y, Z	−59	−56	−55	−53	−52	−43	−33	−32	−33	−31	−32
Value of mean of main neutral batch, if X, Y, Z remained constant	+0	−3	−4	−6	−7	−16	−26	−27	−26	−28	−27
Value of mean corrected for slight aging of X, Y, Z	+0 $=E_6$	−3 $=E_7$	−4 $=E_8$	−6 $=E_9$	−7 $=E_{10}$	−17 $=E_{20}$	−28 $=E_{38}$	−29 $=E_{39}$	−28 $=E_{40}$	−30 $=E_{41}$	−29 $=E_{42}$

NOTE:—*Results are given in microvolts.*

**This column corresponds to Table IV.*

***This column corresponds to row 5, Table VII.*

The calculation of τ is now a simple matter and Table X shows the results for this case. It will be seen on inspection, that for this magnitude of τ and for these differences in time, an error in any of the means of over one microvolt will lead to a large change in the estimate of τ, hence the need for more cells or longer time, for the best determination of this constant.

Having taken $B = -39.4$ from the work of the present writers, and having found $\tau = 2.0$ (Table X), it remains to find A. If readings are referred to the initial reference mean

$$E_6 = A - 39.4 \log_{10}(6+2.0) = 0$$

therefore

$$A = 39.4 \log_{10} 8.0 = 35.6$$

and

$$E_M = 35.6 - 39.4 \log_{10}(M+2.0)$$

which (pending any necessary correction as a result of more extended observation) enables the past and future readings of this group to be correlated.

If it is desired to express the readings in terms of those of the other laboratory, quoted in Table VIII, the equation would be

$$E_M = 28.6 - 39.4 \log_{10} (M + 2.0)$$

in which A has been changed by -7. It is thus possible to keep track of other laboratories with high precision, and subsequent exchanges will indicate the relative changes, if any, in their reference means. If it is desired to recapture a former important measurement which had been referred to a given batch of cells, it is merely necessary to determine the aging constants in this way.

TABLE X

DETERMINATION OF τ

$E_x - E_y$	Differences found in Table IX in microvolts	τ calculated from $E_x - E_y = 39.4 \log_{10}\left(\frac{x+\tau}{y+\tau}\right)$ in months
$E_6 - E_{38}$	28	1.8
$E_7 - E_{39}$	26	2.0
$E_8 - E_{40}$	24	2.5
$E_9 - E_{41}$	24	1.3
$E_{10} - E_{42}$	22	2.2
$E_6 - E_{20}$	18	2.3
$E_{20} - E_{42}$	12	1.8
Mean value of $\tau = 2.0$ months		

If only the cells and the observations quoted in this illustration were available, it is probable that any given mean e.m.f. estimated in this manner within many years of these observations, would not be in error by as much as six microvolts. With auxiliary groups and longer observations, it is considered

TABLE XI

ILLUSTRATING THE HIGH PRECISION OF THE AGING EQUATION

Age in months	1	3	6	10	15	30	50	120	230	240
Mean for seven cells calculated from $E_M = 38.0 - 39.4 \log_{10}(M + 0.75)$	+28	+15	+5	−3	−9	−21	−30	−45	−56	−57
Obtained experimentally with aid of newer cells, and acid cells	+27	+14	+8	−3	−14	−19	−29	−46	−53	−57
Difference in millionths of a volt	−1	−1	+3	+0	−5	+2	+1	−1	+3	+0
Mean for thirty cells calculated from $E_M = 29.5 - 39.4 \log_{10}(M + 0.75)$	+20	+7	−3	−11	−18	−29	−38	−53	−64	−65
Obtained experimentally with aid of newer cells, and acid cells	+20	+6	−3	−11	−19	−29	−38	−53	—	—
Difference in millionths of a volt	+0	−1	+0	+0	−1	+0	+0	+0	—	—

NOTE:—*Results are given in microvolts.*

that this can be reduced to one or two microvolts, as indicated in the evidence presented in a former paper on this subject (9).

In order to illustrate the exceptional precision possible when a large number of readings is taken, some sample observations on a small batch of seven cells over a period of 20 years, and on a large batch of 30 cells over 10 years, are shown in Table XI*. Each figure is the mean of the actual readings for all the cells of the batch indicated, and in each case the mean of several readings for each cell is also involved. The initial reference mean was recaptured for each date by at least two independent methods.

Some Notes and Relations

The following is a summary of relations determined or verified at this laboratory, which are useful in the recapture and correction of old measurements, in the maintenance of a standard volt, or in the ordinary use of standard cells.

(*a*) *The Electromotive Force of the International Mean Weston Normal Cell in Semi-absolute Volts, in Absolute Volts, and in International Volts*

The electromotive force of the mean Weston normal cell was found to be 1.01809 ± 0.00003 semi-absolute volts at 25.00° C. (7) and using the temperature formula

$$E_t = E_{20} - 0.000040\ (t-20) - 0.0000009\ (t-20)^2$$

this gives 1.01831 ± 0.00003 semi-absolute volts at 20.00° C.

The recent work on aging leads to a negligible correction of -4 microvolts, which is beyond the reliable significant figures of our absolute measurement.

Assuming, as recommended by Birge (1, see page 18), that

1 semi-absolute volt $= 1.00051 \pm 0.00002$ absolute volts

$= 1.00005 \pm 0.00005$ international volts

we find the following values for 20.00° C.:—

1.01831 ± 0.00003 semi-absolute volts

or 1.01882 ± 0.00005 absolute volts

or 1.01836 ± 0.00008 international volts.

These figures suggest after comparison with other absolute measurements that the international volt is probably nearly equal to the semi-absolute volt used here, that is, that the international ampere more nearly equals the absolute ampere than is indicated by the correction of five parts in 100,000 applied above (4). Otherwise the validity of assuming that the international volt now defined in terms of the Weston cell (taken as 1.01830 at 20.00°C.) is equal to the original international volt, must still be questioned.

It should be noted that this value of 1.01831 ± 0.00003 semi-absolute volts involves the value of the international ohm deduced from (a) McGill standards, and (b) comparisons with the Bureau of Standards at Washington.

**The cells in the first batch are cells IV, VI, A, B, C5, N and Q3. The cells in the second batch are P1, P2, P3, P4, P5, Q2, Q3, Q4, Q5, Q6, IV, VI, VIII, A, B, C2, C3, D, N, O, 60, 63, 64, 66, 67, 68, 69, 71, 78 and 79.*

It is unfortunate that these absolute measurements are still uncertain to about 50 parts in a million when the reproduction and maintenance of an arbitrary volt is possible to within one or two parts in a million.

(*b*) *The Electromotive Force of Standard Acid Weston Cells*

The mean value of the acid cell (made with the 10% amalgam, and using $N/10$ H_2SO_4), at the age of six months at 25.00° C. was found to be

(International mean for neutral cells) $-(66 \pm 2)$ microvolts.

This fell to -69 ± 2 microvolts in five years. In individual cells, one year old, values as low as -76 and as high as -59 were observed as extreme cases, in a group of 20 carefully prepared cells in which ingredients from various sources were represented. These extremes were, however, exceptional; in the best case observed the mean deviation from -66 in a picked group of ten cells on a given date was only two microvolts, and a year later only three microvolts.

This decrease of -66 microvolts is greater if the acid used is of higher normality. According to data in the International Critical Tables it would appear that the decrease could be represented approximately by $22-880n$ microvolts, (where n is the fraction of normality) for values of n from about 0.07 to 0.75. An expression of the type

$$1950 \log_{10}(1-0.74n)$$

includes the case $n=0$, but may fail at higher concentrations. The observations described here were confined to the case $n=0.10$.

(*c*) *The Ratio of the Electromotive Force of the Standard Neutral Weston Cell to that of the Standard Clark*

At 25.00° C.,

$$\frac{\text{Weston}}{\text{Clark}} = 0.716956 \pm 0.000004,$$

where each is assumed to have the international mean value as defined in 1908.

(*d*) *The Relation Between Clark Cells "Set Up," According to the Old Board of Trade Specifications, in Test Tubes, and the Standard Clark Cell, International Mean*

At age one to six months, at 25.00° C, for the mean of a batch of five,

("Test-tube" Clark) = (Standard Clark) $+(320 \pm 50)$ microvolts.

The deviation ± 50 might occasionally be as high as ± 150 for one cell.

If the test-tube cells were made as far as possible according to the later, more detailed specifications for the H-type, this deviation would doubtless be reduced.

(*e*) *The Relation Between the Test-tube Inverted Type of Weston Cell and the Standard Neutral Weston Cell, International Mean*

At age one to six months, at 25.00° C., for the mean of a batch of five,

(Test-tube inverted Weston) = (Standard Weston) $+(380 \pm 100)$ microvolts.

This result was based on only six specially prepared cells and a collection of older cells of somewhat uncertain history. If the modern specifications were followed in detail as far as they are applicable to this type, it is probable that

the deviation would be reduced. These cells tend to develop high resistance very readily.

(*f*) *The Effect on the Electromotive Force of Ignoring the More Tedious Processes of Construction Required by Modern Specifications*

If the ingredients are taken as furnished by the average manufacturer, and rapidly assembled into a cell, it is rarely found that such a cell will differ from the reference mean of standard cells by more than 500 microvolts. Thus for all work requiring an accuracy not better than five parts in 10,000, the standard specifications may be ignored safely. This does not mean that the cells may be constructed carelessly, because in the hands of an inexperienced worker (by the inclusion of added impurities such as might occasionally happen without assuming any extreme accident) divergences of several thousand microvolts will sometimes occur. Quite often these freak cells are as constant as the good cells, and if previously measured may be of equal use in the laboratory; as a general rule, however, they take several years to reach a steady state.

With intermediate modifications as described by Bronson and Shaw (2, 3) much time is saved, and the divergences are usually within 100 microvolts; furthermore, the cells in this class appear to be as constant (and to have the same value of the constant B in the aging equation) as the full-fledged standard neutral cells.

(*g*) *The General Aging Equation*

The most general form of the aging equation for neutral cells has been found on certain theoretical assumptions to be

$$E = E_0 - 17.1 \log_e \left(\frac{1 - e^{-0.0006(t+\tau)}}{1 - e^{-0.0006\tau}} \right)$$

where E is the total e.m.f., E_0 is the total e.m.f. when $t = 0$, and τ varies from about 0.5 up to 25 for various cells. (In this investigation the majority of the values of τ were a little less than unity.)

The value of k (namely, 0.0006, above) is uncertain but owing to its small magnitude and the type of formula, it is arithmetically unimportant. This formula has been shown to have a reasonable theoretical basis, but for general usage over periods up to twenty years the following approximate formula which follows from the above since k is small, is more convenient:—

$$E_M = A - 39.4 \log_{10}(M + \tau)$$

where M is the age of the cell in months and E_M is the difference in voltage from the initial reference mean.

(*h*) *Relations Between Rates of Aging and Age of Weston Cells*

TABLE XII

RATES OF AGING IN MICROVOLTS PER MONTH AT VARIOUS AGES FOR NEUTRAL WESTON CELLS WITH TYPICAL VALUES OF τ

τ	Cells 0 months old	Cells 3 months old	Cells 6 months old	Cells 1 year old	Cells 2 years old	Cells 5 years old	Cells 10 years old	Cells 20 years old
0.5	34.2	4.9	2.6	1.4	0.70	0.28	0.14	0.07
0.75	22.8	4.6	2.5	1.3	0.69	0.28	0.14	0.07
1.0	17.1	4.3	2.4	1.3	0.68	0.28	0.14	0.07
5.0	3.4	2.1	1.6	1.0	0.59	0.26	0.14	0.07
10.0	1.7	1.3	1.1	0.78	0.50	0.24	0.13	0.07
15.0	1.1	0.95	0.81	0.63	0.44	0.23	0.13	0.07
20.0	0.85	0.74	0.66	0.53	0.39	0.21	0.12	0.07
25.0	0.68	0.61	0.55	0.46	0.35	0.20	0.12	0.06

(*i*) *International Comparisons*

A series of exchanges extending over twenty years (9) have shown an average difference of zero from the Bureau of Standards at Washington for eight exchanges, with a maximum divergence of six microvolts, while for five exchanges with the National Physical Laboratory at Teddington, the average difference was one microvolt, with a maximum of eight. It is thought that these differences are due to the disturbances of the transported cells, and to the fluctuations in the reference means. Although the reference means at the leading standards laboratories have been maintained with high precision through the investigation of large numbers of cells and the frequent selection of new cells (with what we call high values of τ, that is, with slow aging), it is thought that these fluctuations could be eliminated, and that only a few batches of cells would be necessary. If batches are selected according to the directions of this paper, and their aging equations determined, it is considered that a definite international reference mean could be recaptured when required to within one part in a million.

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